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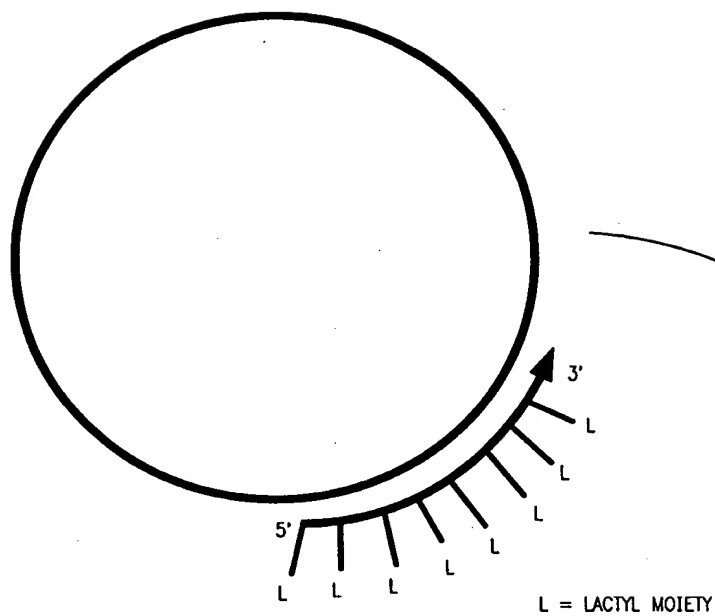
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(A)



(B)

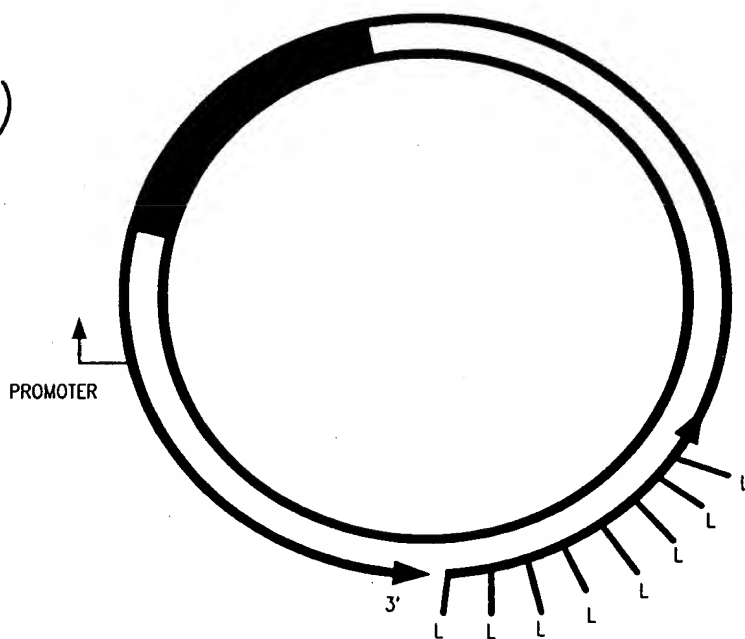


FIG. 1

ATTACHMENTS OF LIGANDS THROUGH PRIMER REGION



(A)

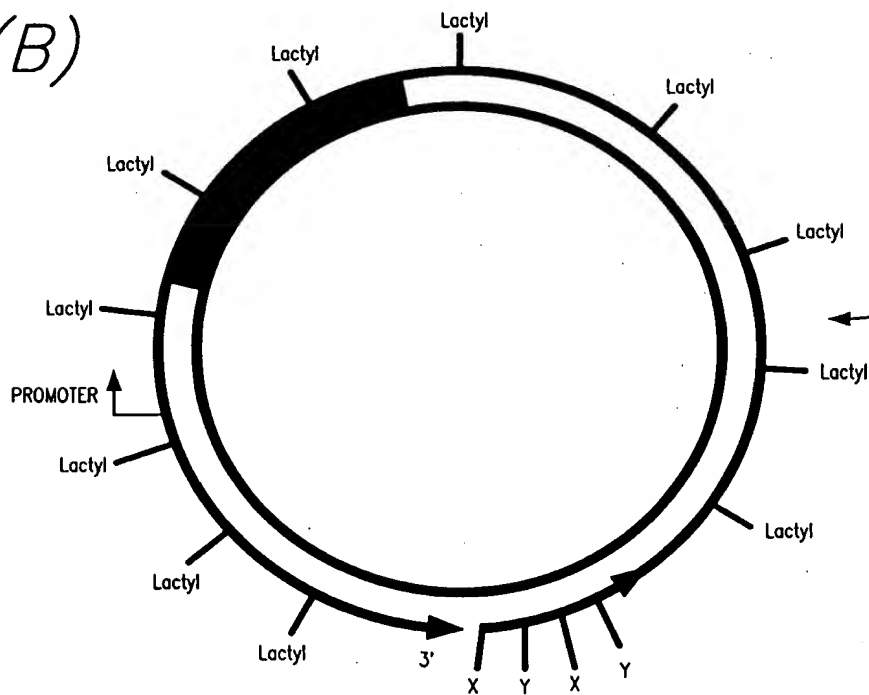


FIG. 2

ATTACHMENT OF LIGANDS BY INCORPORATION OF MODIFIED NUCLEOTIDE PRECURSORS

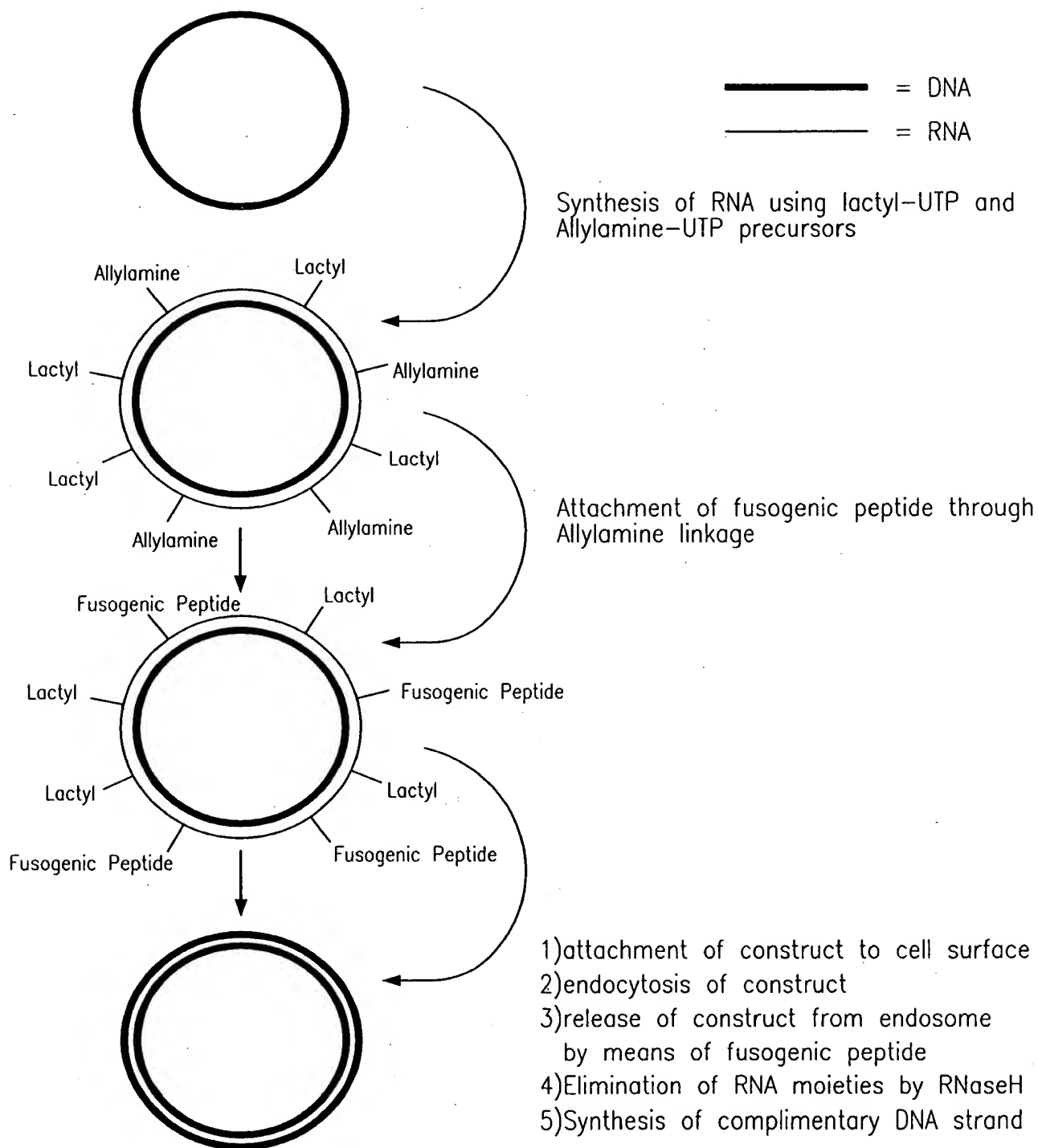


FIG. 3

Incorporation of Ligands through Modified Ribonucleotides

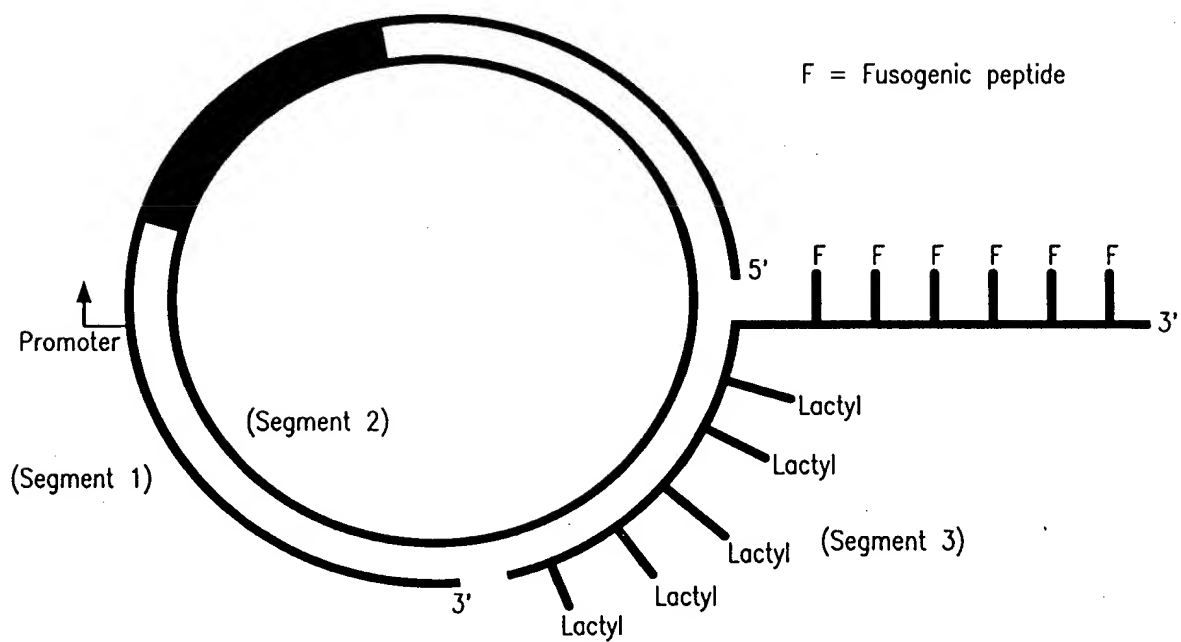


FIG. 4

Attachment of Ligands through a 3' tail

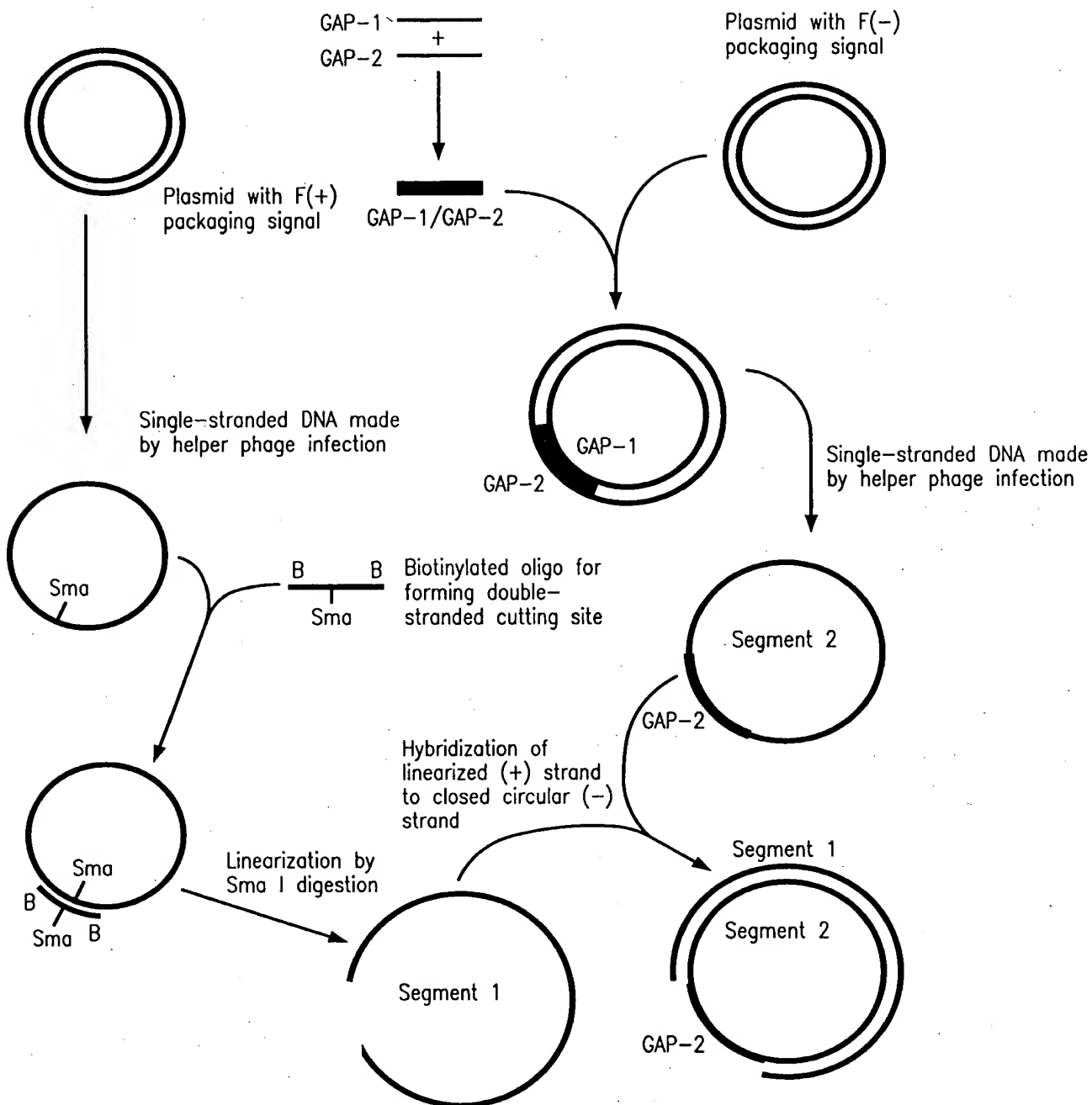


FIG. 5

Preparation of Gapped Circle

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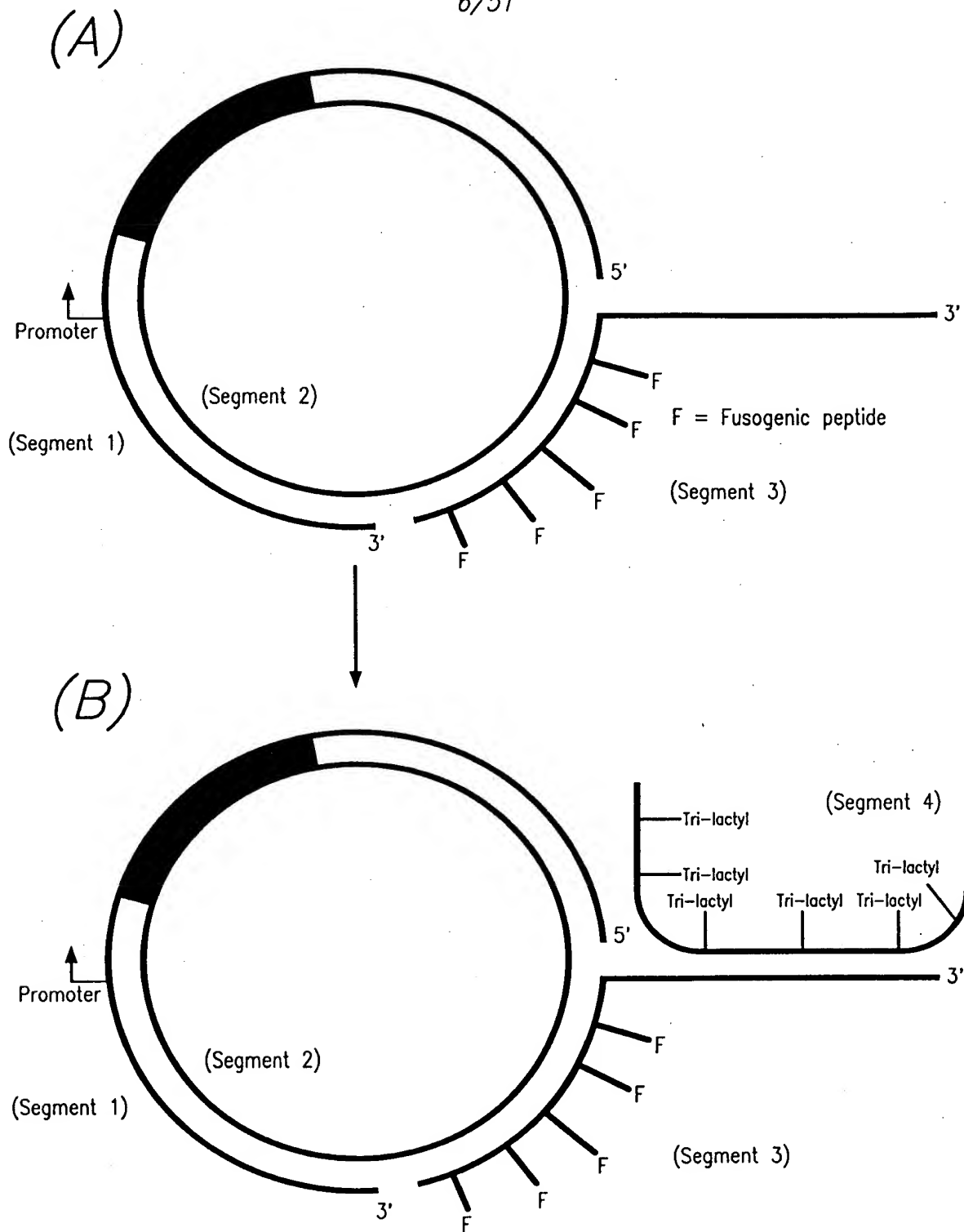


FIG. 6

Attachment of Ligands through hybridization to a 3' tail

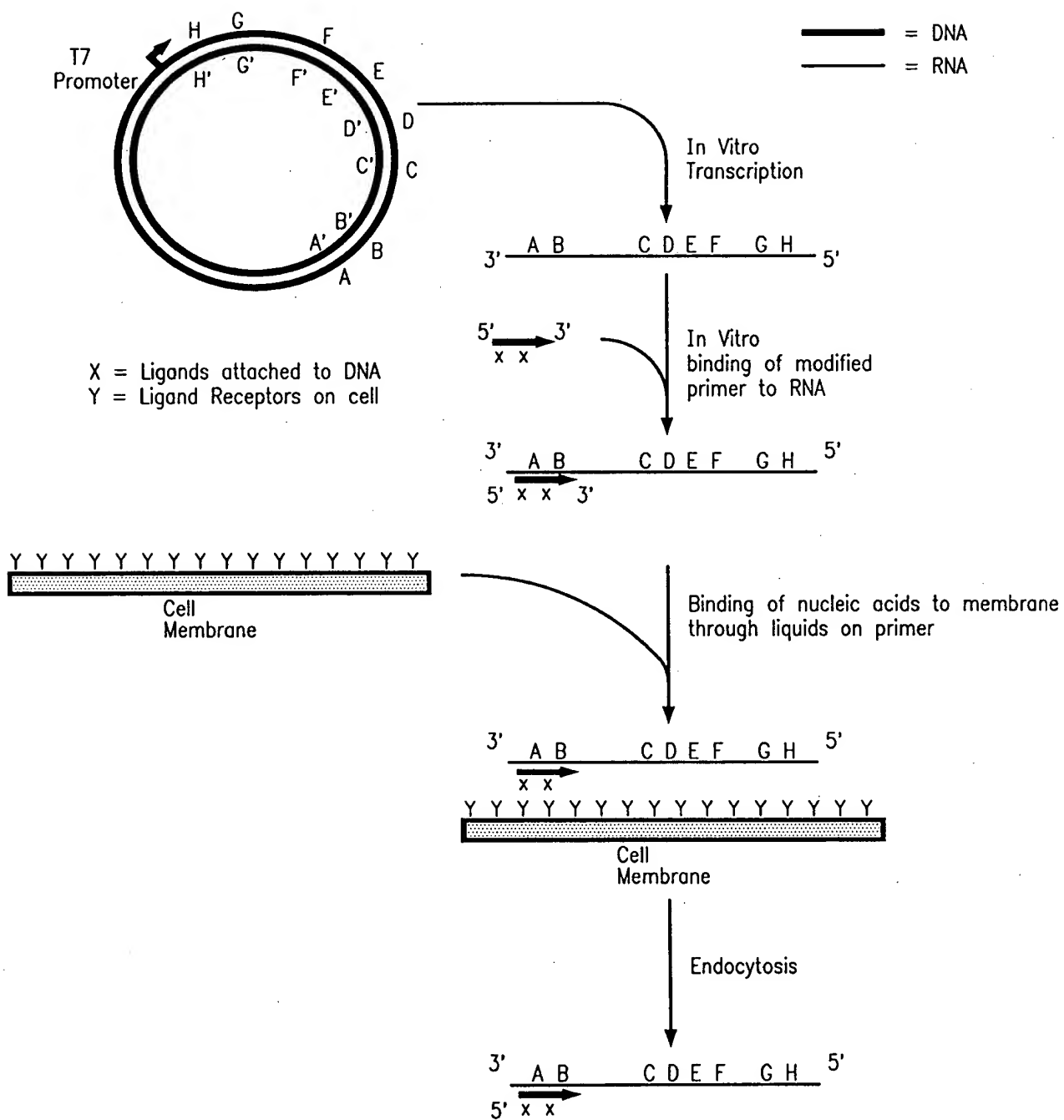


FIG. 7

RNA with Ligands on Primer

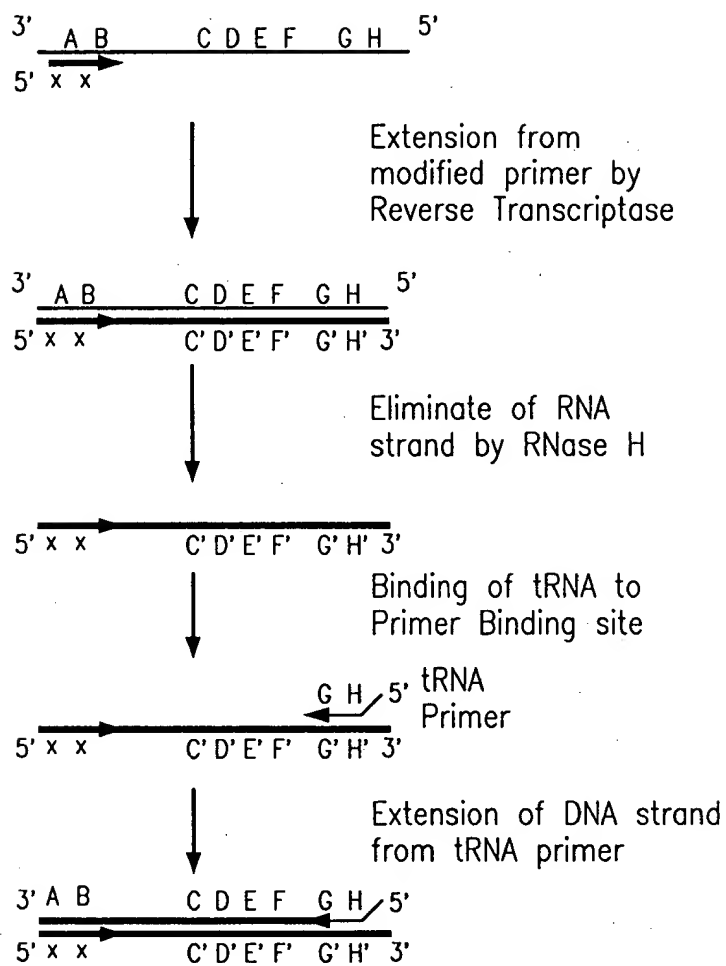


FIG. 8

RNA with Ligands on Primer (Continued)

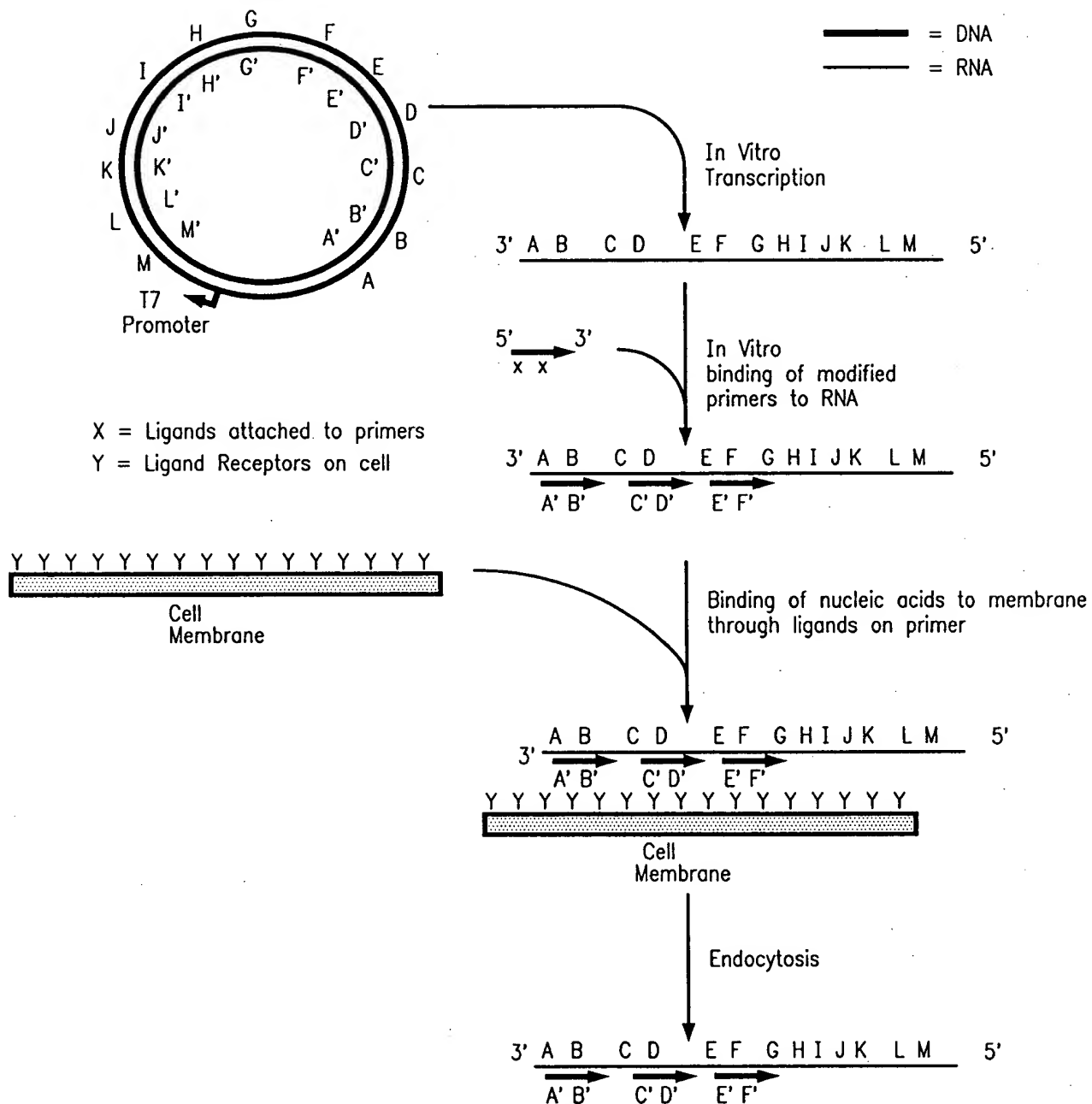


FIG. 9

RNA with Ligands on Multiple Primers

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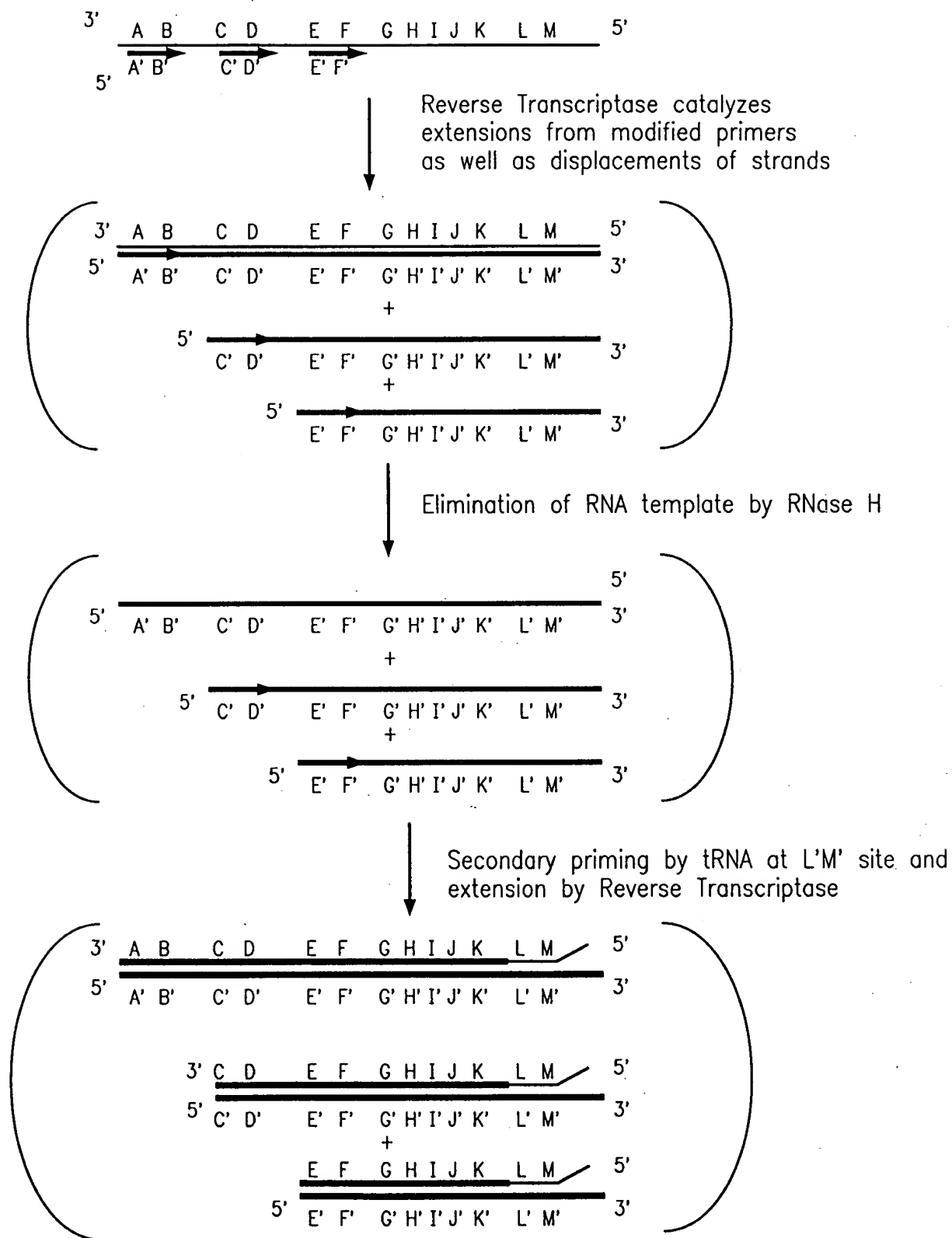


FIG. 10

RNA with Ligands on Multiple Primers (Continued)

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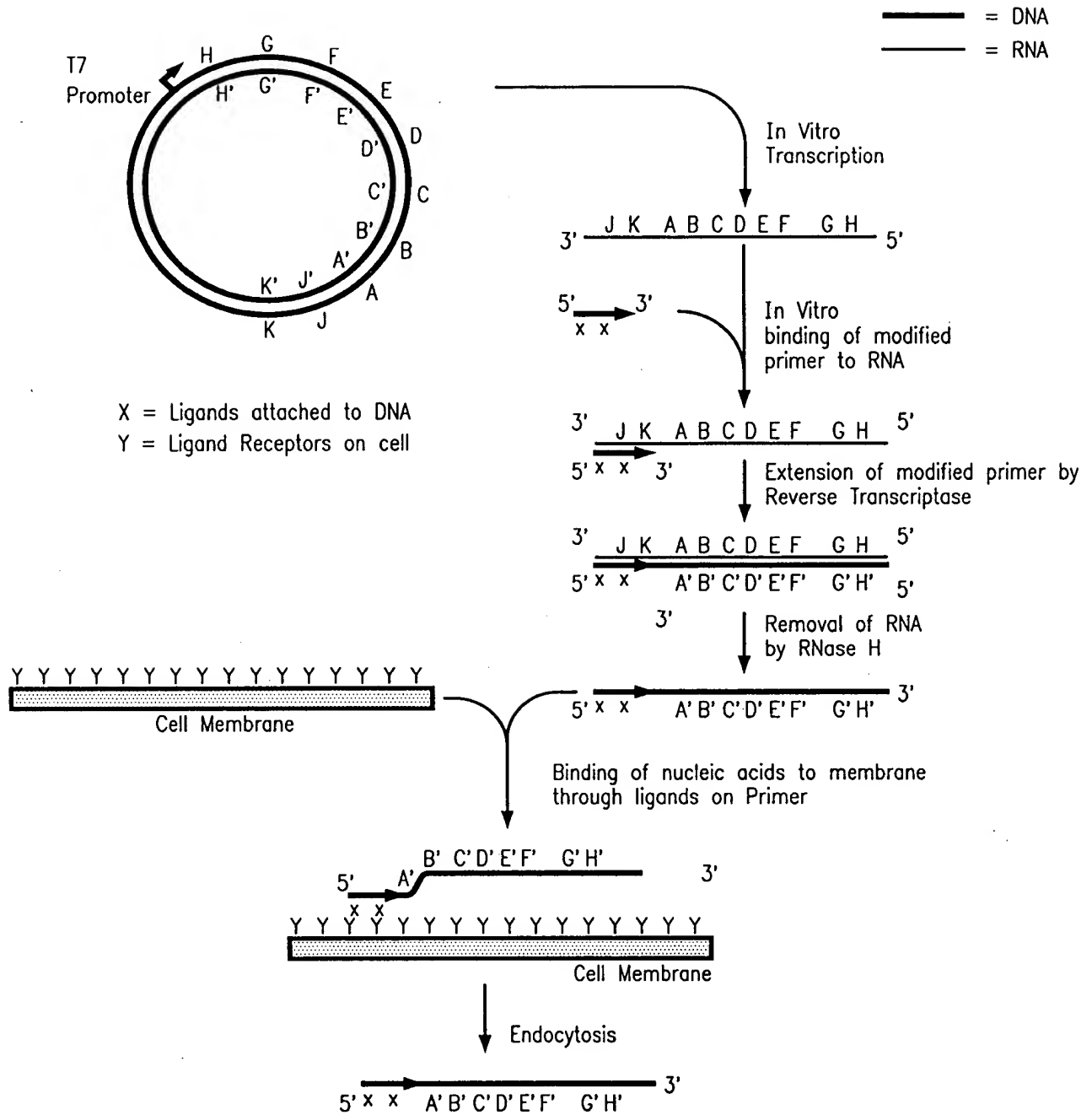


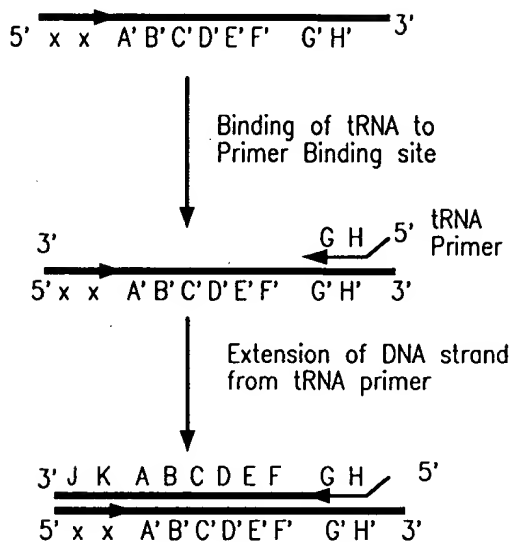
FIG. 11

Single-stranded DNA with attached Ligands

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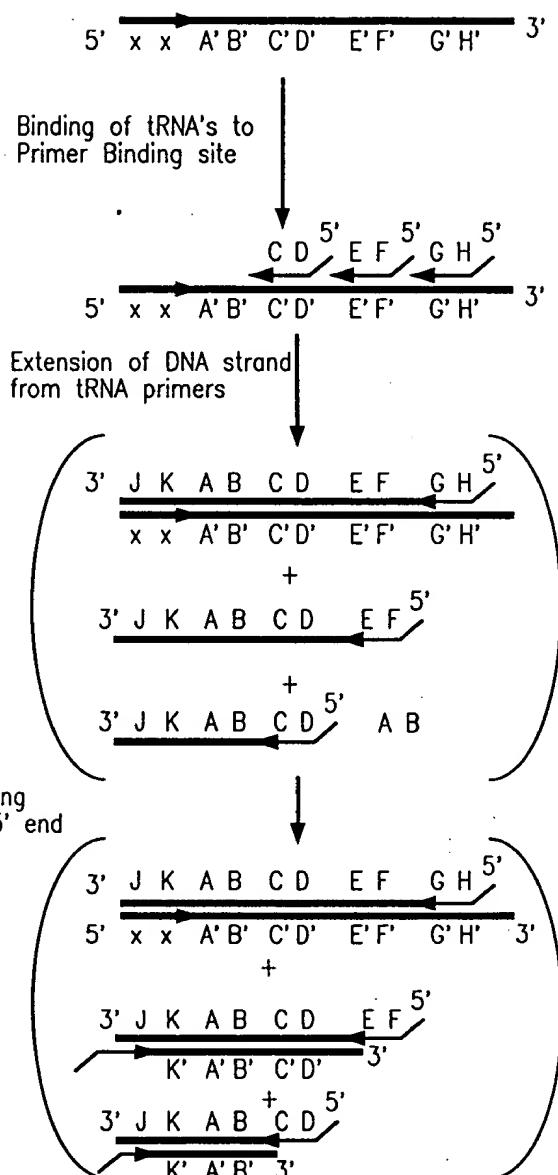
(A)

Presence of a single
tRNA primer site



(B)

Presence of multiple
tRNA primer sites



Synthesis of second strand by binding
of tRNA to Primer Binding site at 5' end

FIG. 12

Single-stranded DNA with attached Ligands (continued)

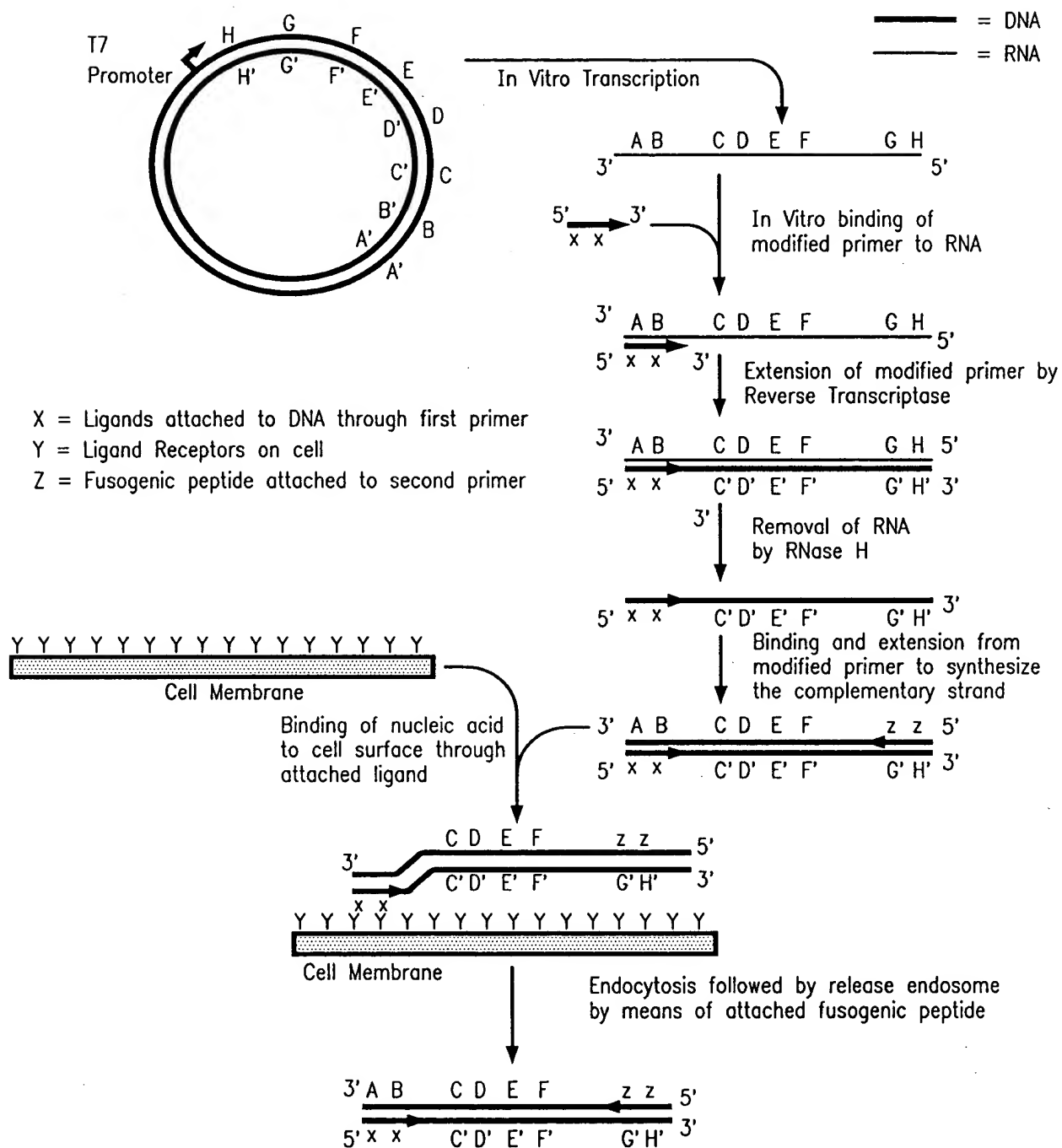


FIG. 13

Linear Double-stranded DNA with attached Moieties on each strand



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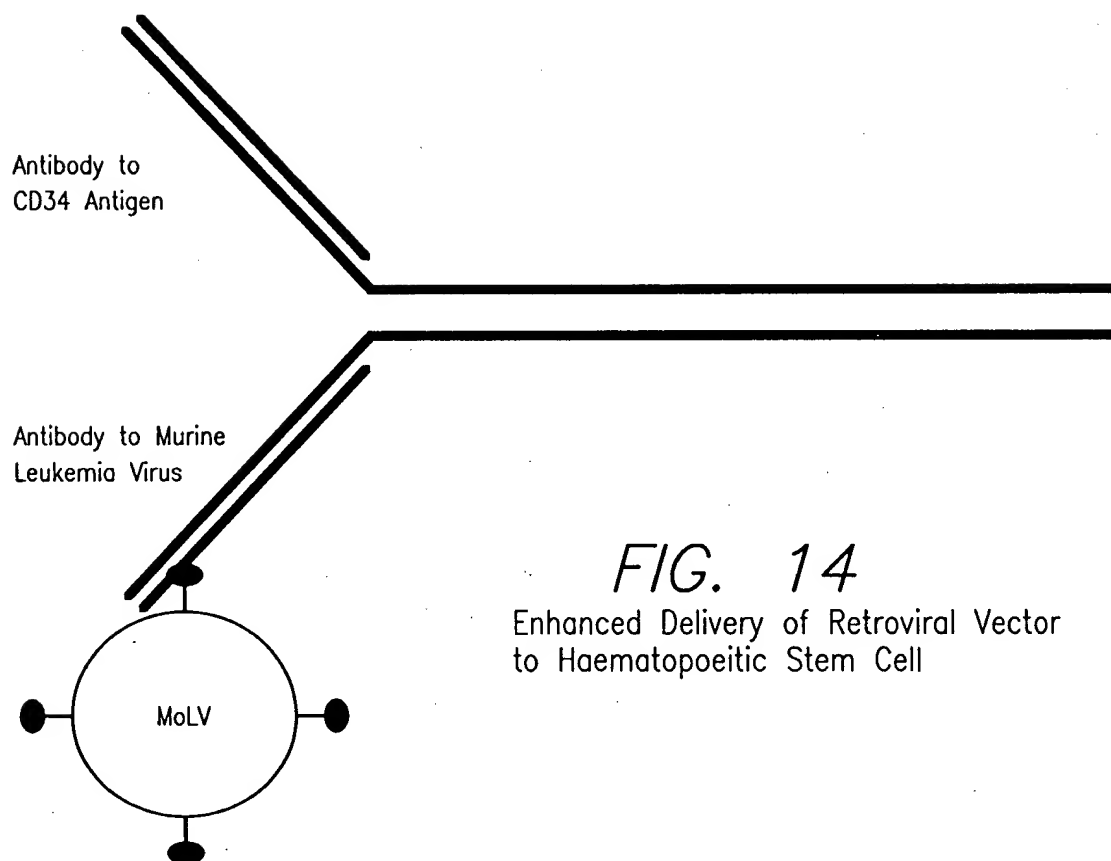


FIG. 14
Enhanced Delivery of Retroviral Vector
to Haematopoietic Stem Cell

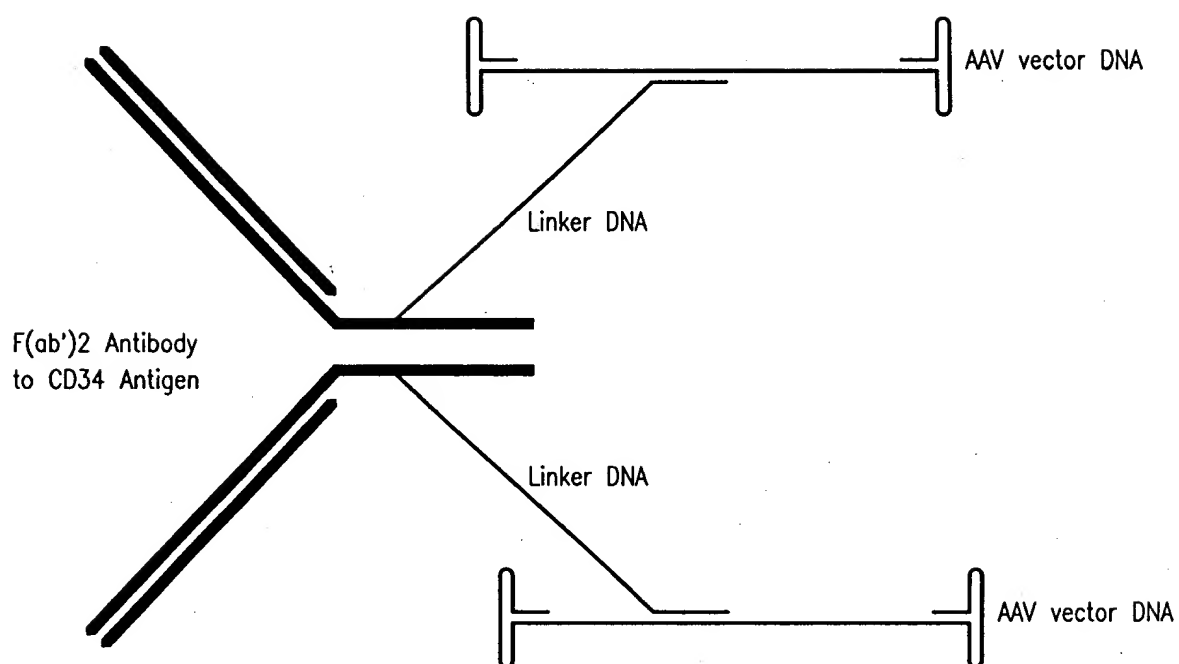
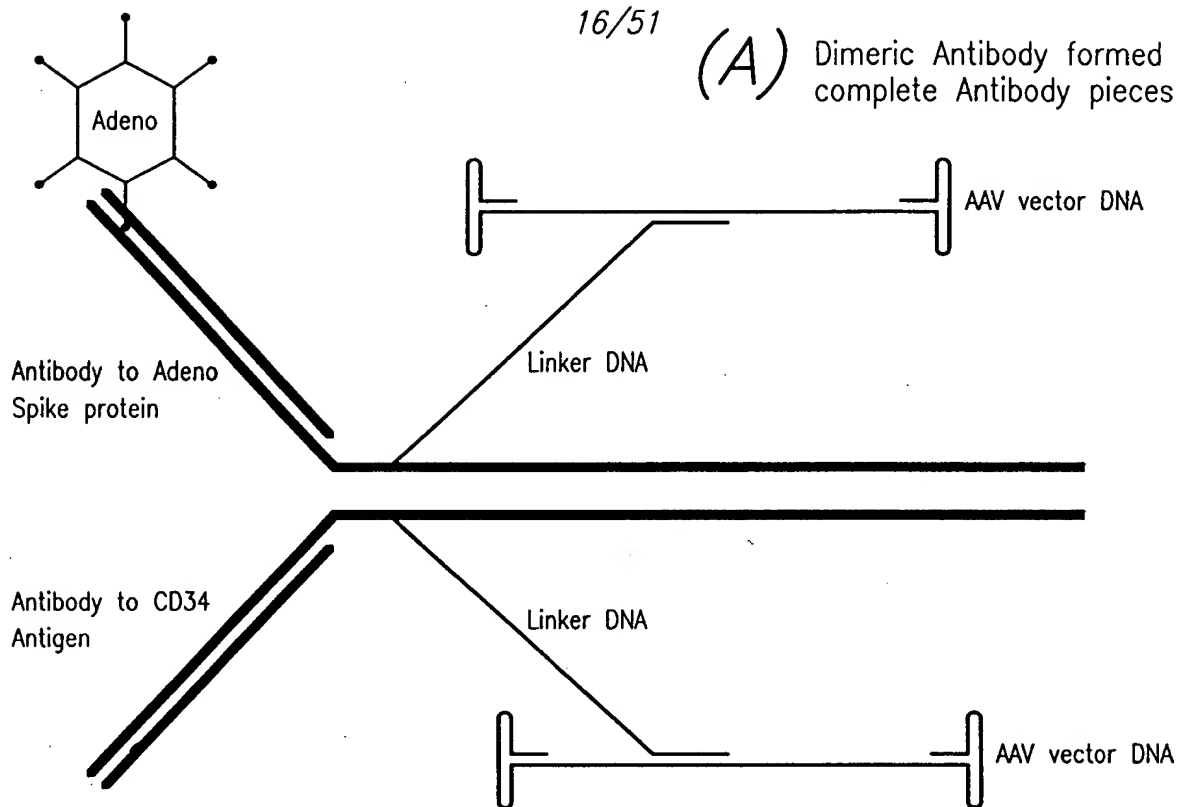


FIG. 15
Enhanced Delivery of Vector
DNA to Haematopoietic Stem Cell

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(A) Dimeric Antibody formed from complete Antibody pieces



(B) Dimeric Antibody formed from F(ab') fragments

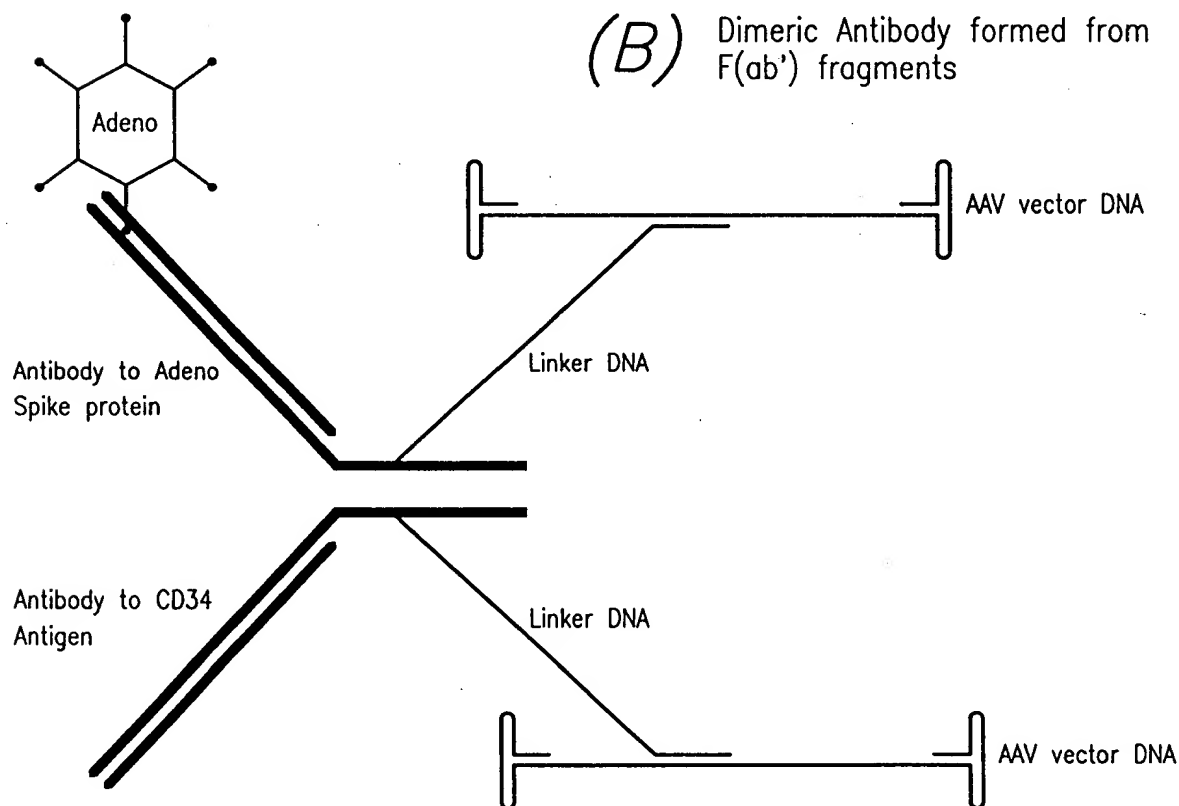


FIG. 16

Covalent Attachment of vector DNA to Dimeric Antibody

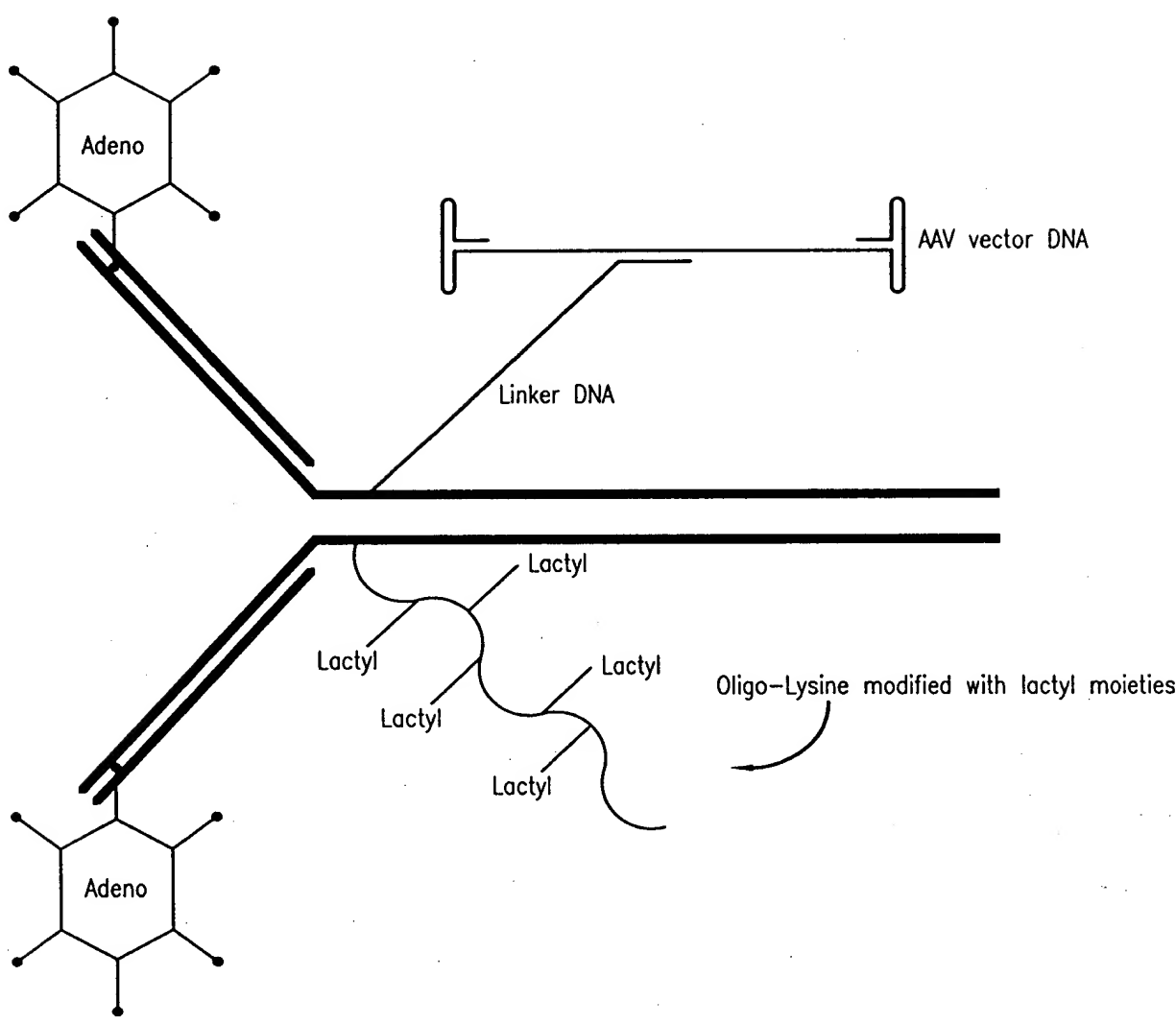


FIG. 17

Covalent attachment of Modified DNA
to a Monovalent Antibody

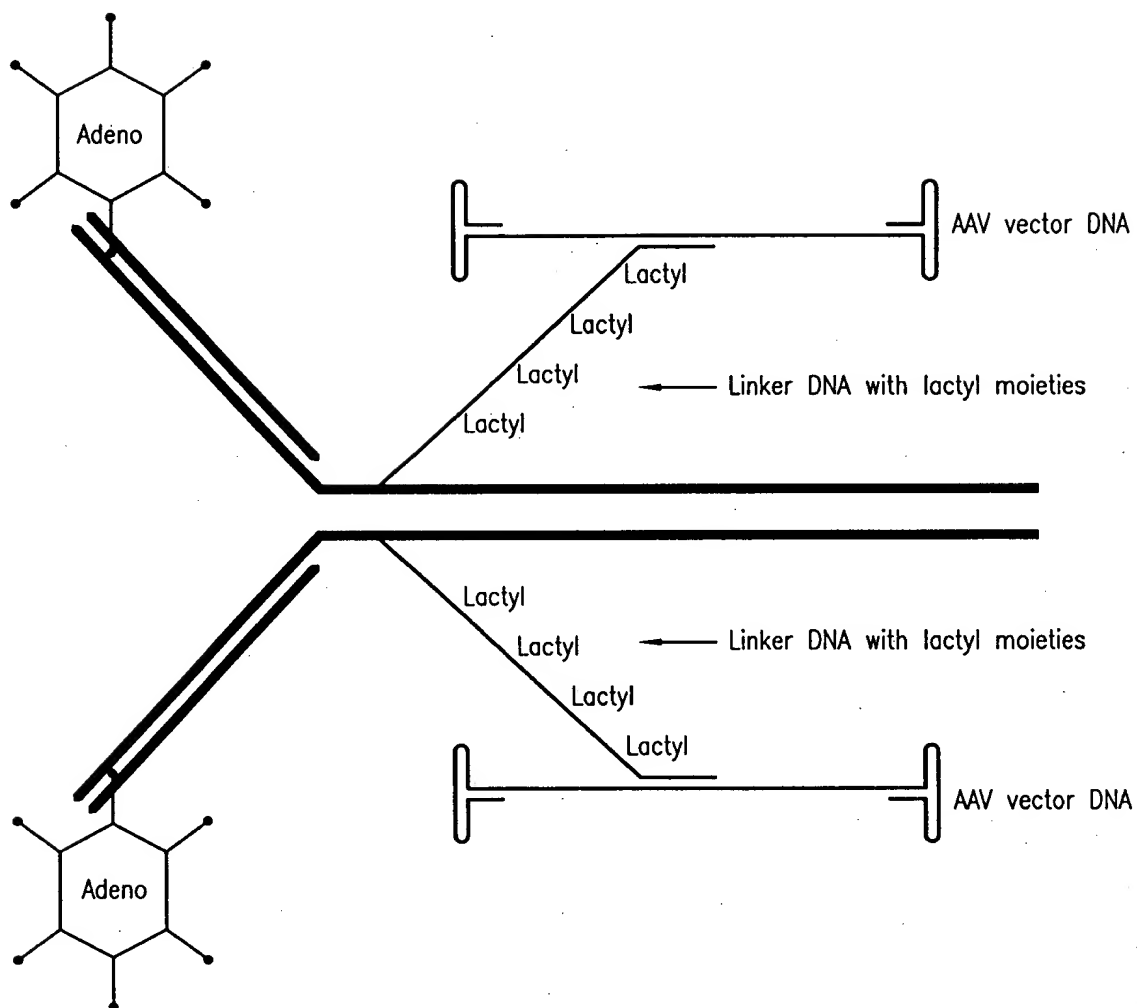


FIG. 18

Modified DNA used as a Binder

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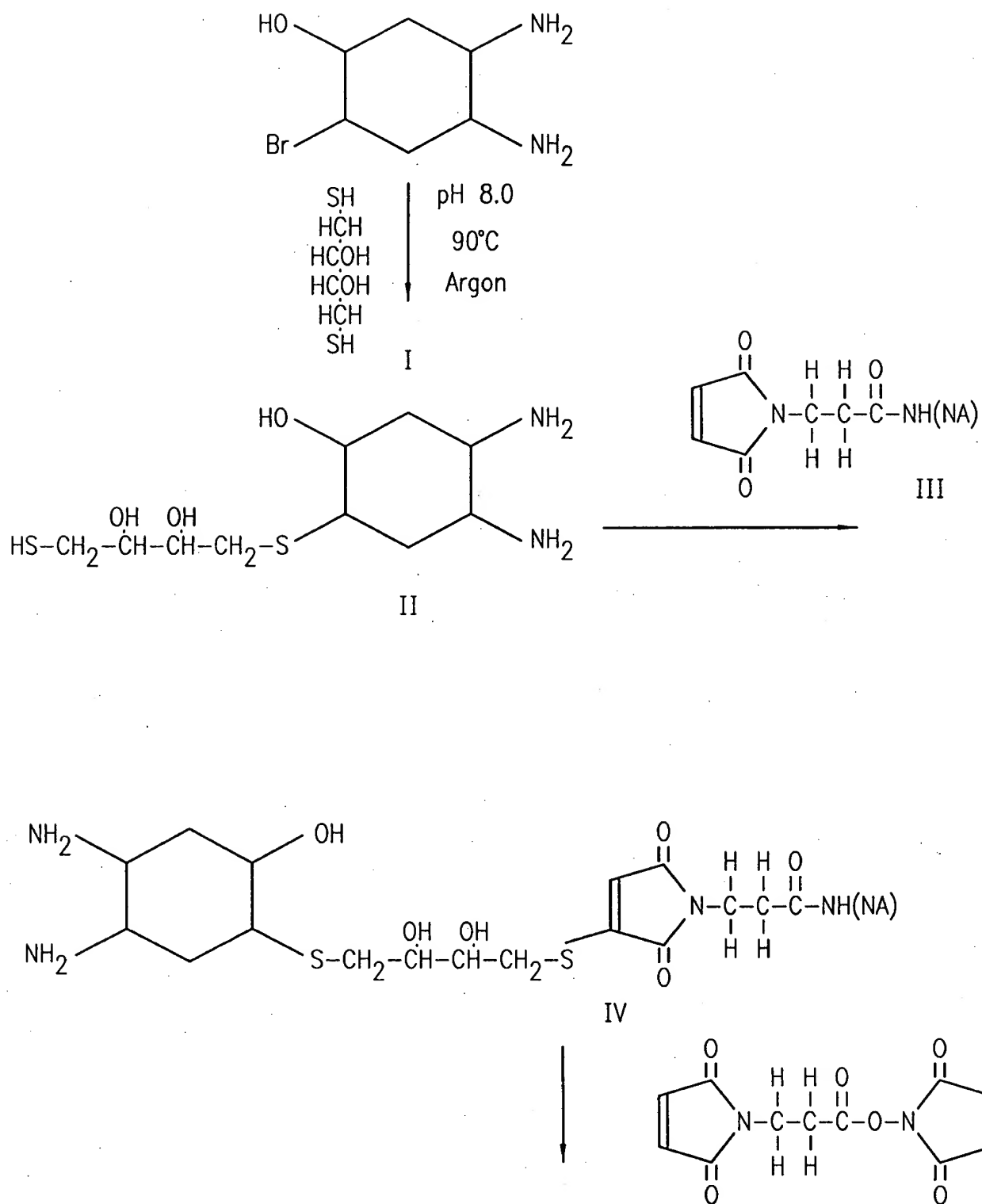


FIG. 19

Synthetic Steps for Creation of Antibodies
 With Nucleic Acid Moieties Attached

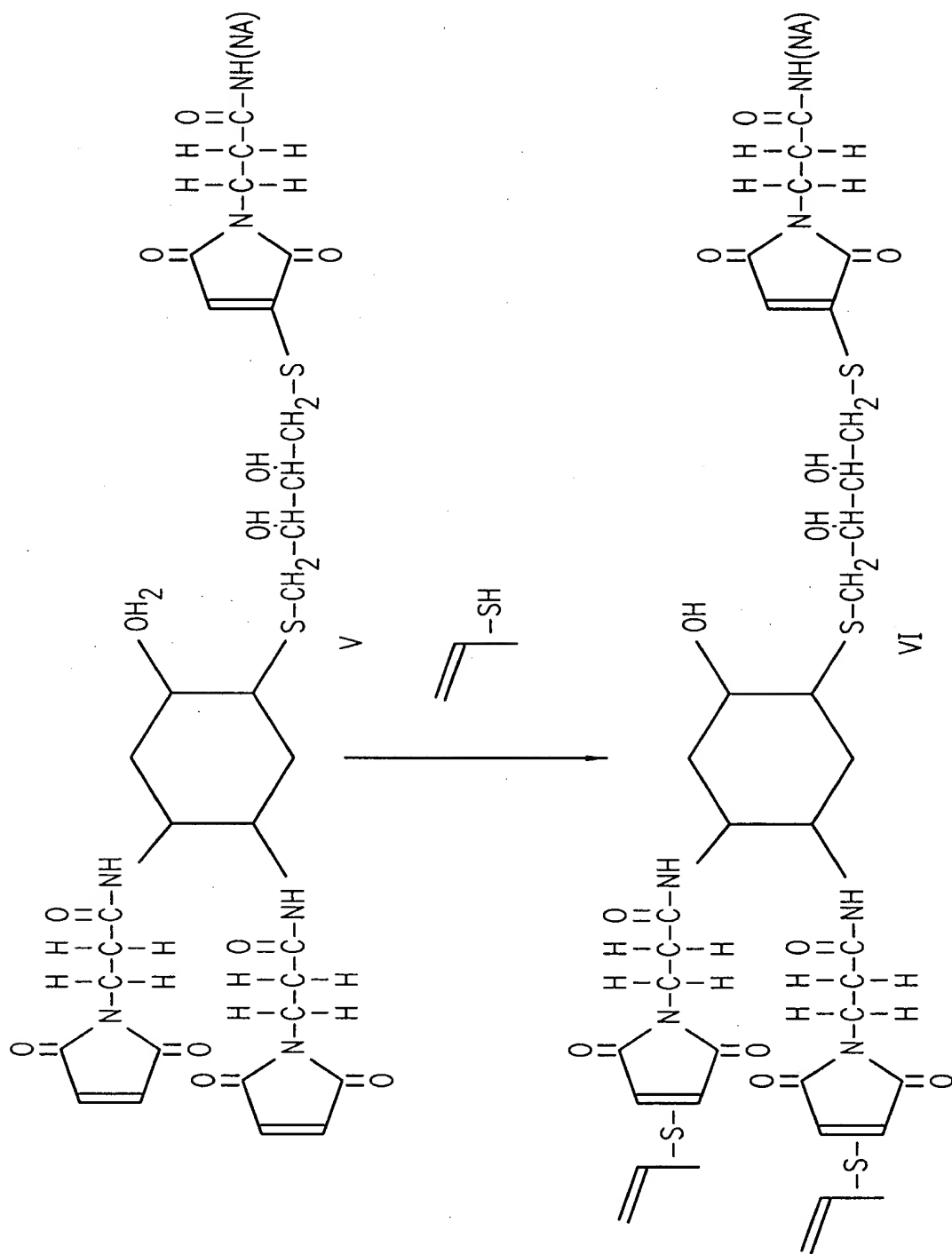


FIG. 20

Continuation of Synthetic Steps

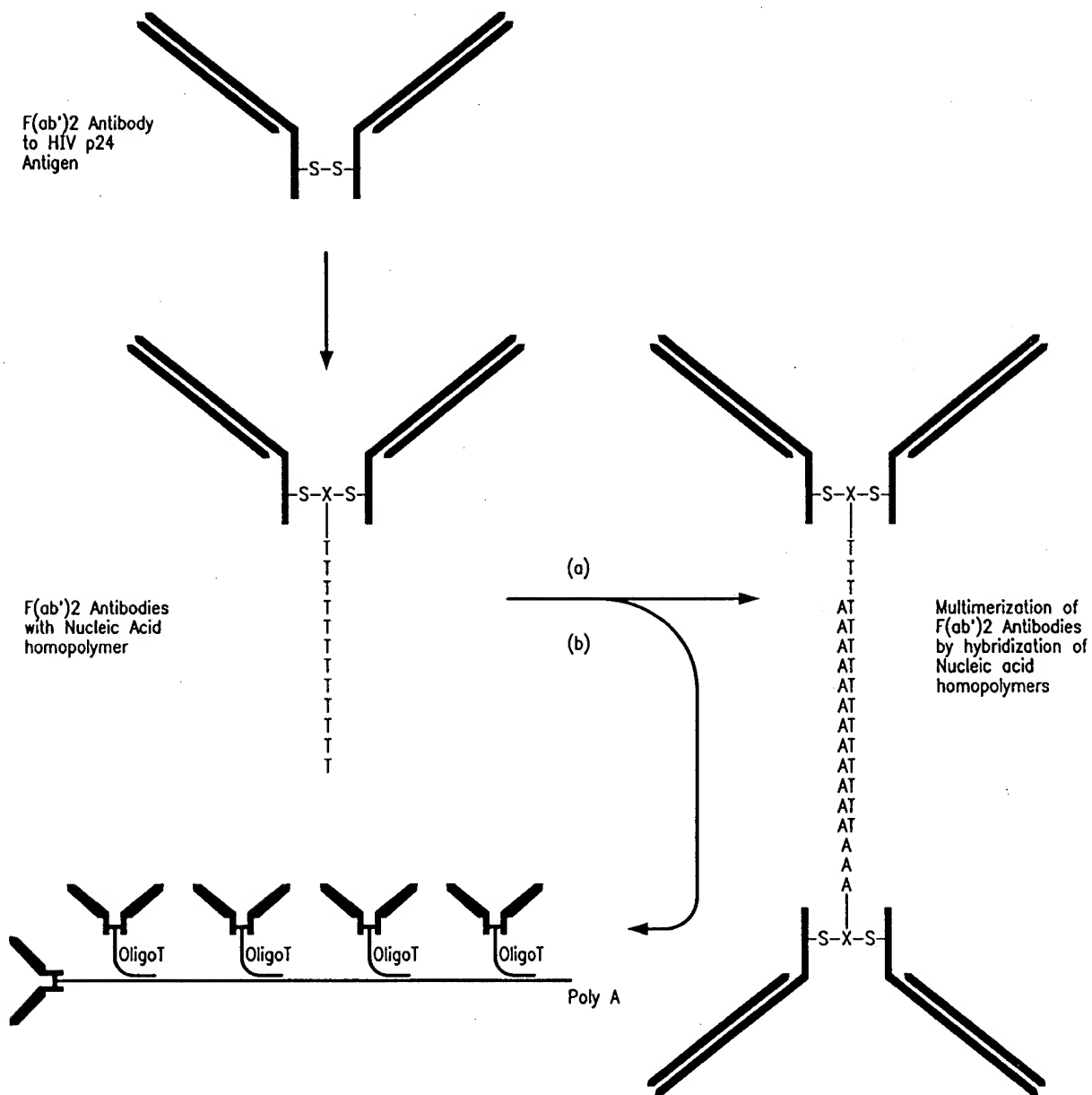


FIG. 21

Enhanced Binding of Antibodies to Antigens by Multimerization

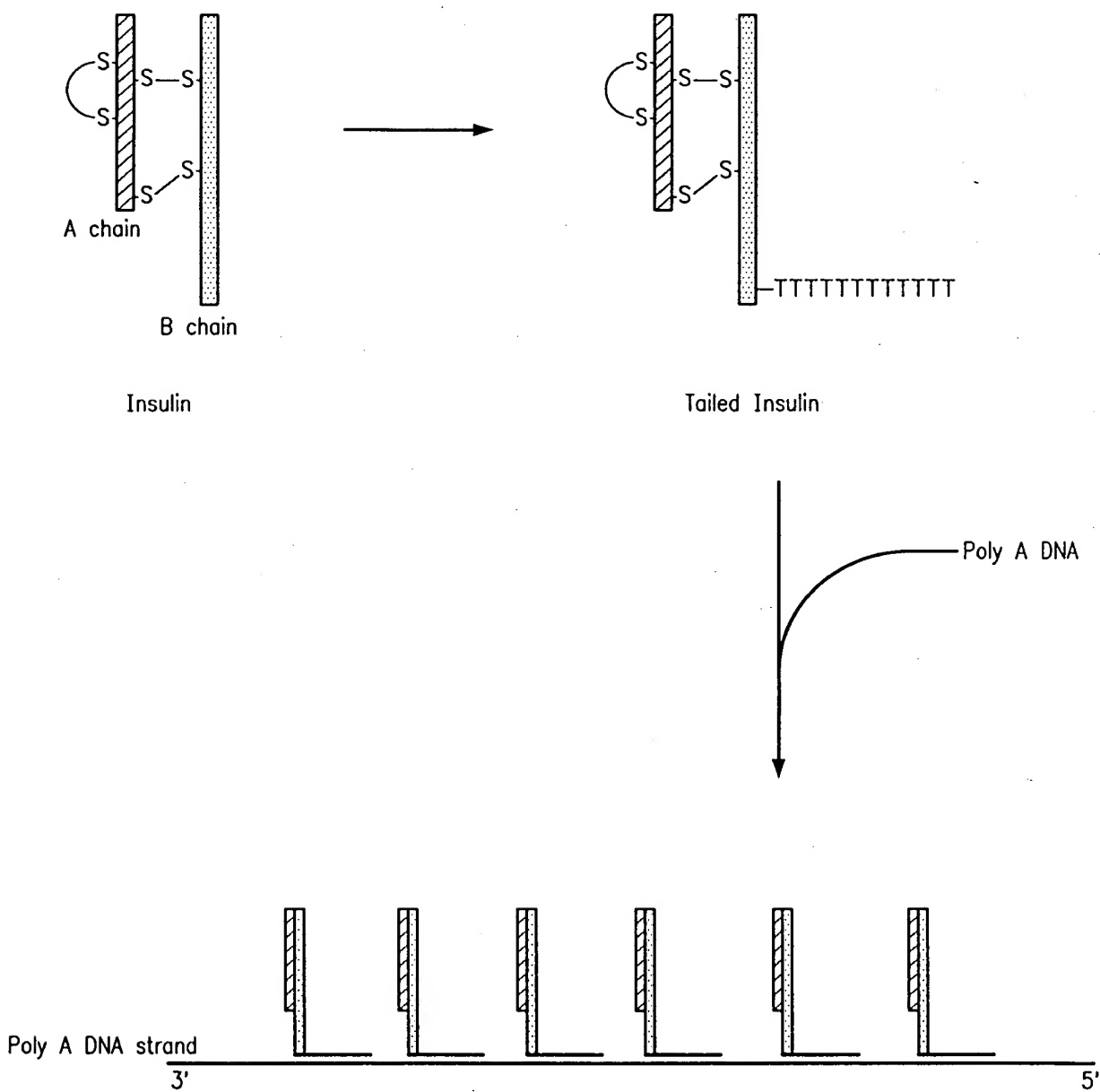


FIG. 22

High Affinity Multi-Insulin Soluble Complex

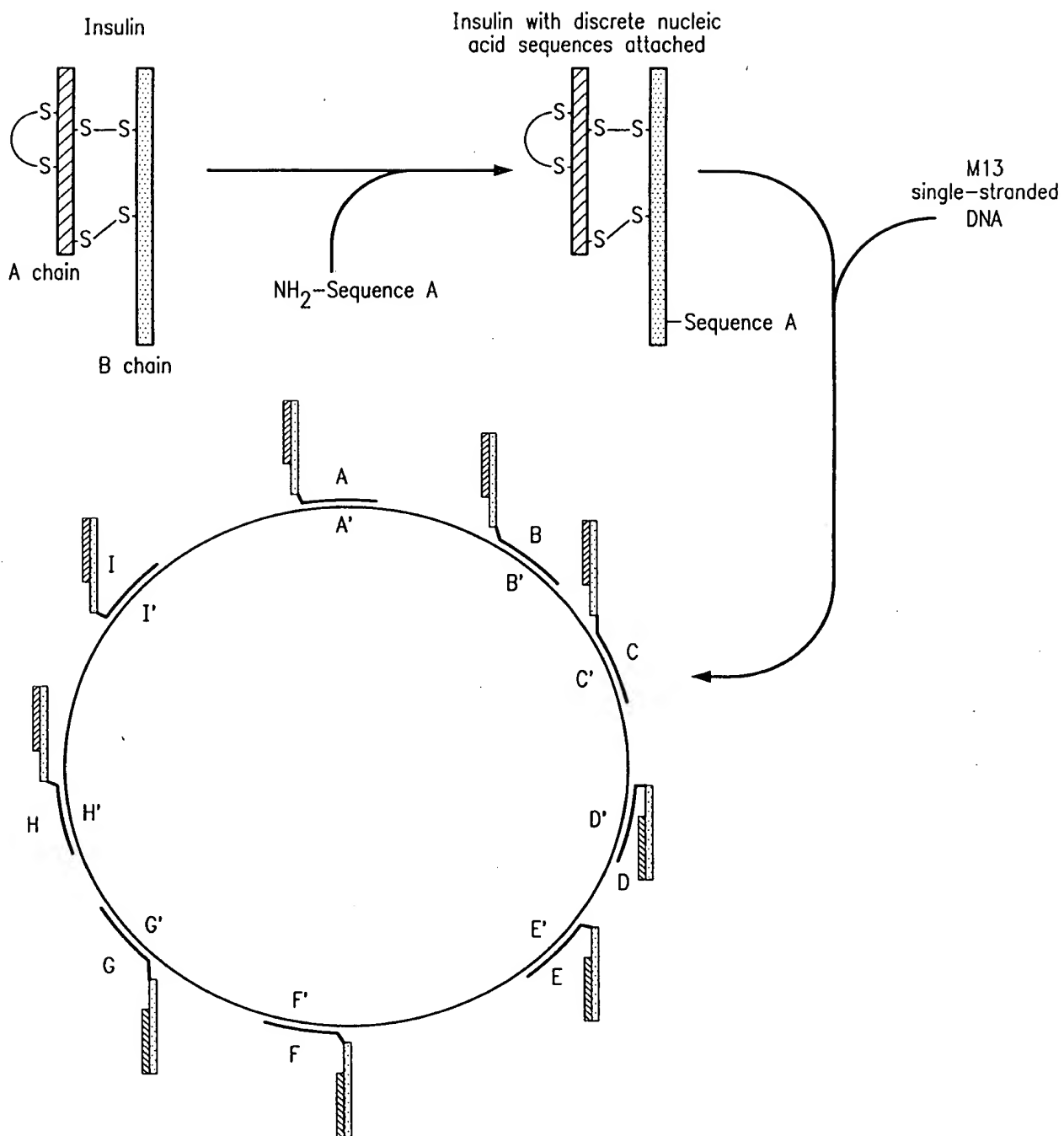


FIG. 23

Multimerization of Insulin molecules by hybridization to discrete Sequences

Intron insertion site
 ↓
 (A) -----TGCTCTCTAAGGGTCTACTC-----
 -----ACGAGAGATTCCCAGATGAG-----
 T7 RNA Polymerase Sequence

Splice Donor Site Splice Acceptor Site
 ↓ ↓
 (B) -----CTCTAAGGTAAATAT - - - - - TGTATTTTAGATTCAA-----
 -----GAGATTCCATTATA - - - - - ACATAAAATCTAAGTT-----
 SV40 Intron Sequence

(C) -----TGCTCTCTAAGGTAAATAT - - - - - TGTATTTTAGGGTCTACTC-----
 -----ACGAGAGATTCCATTATA - - - - - ACATAAAATCCCAGATGAG-----
 Insertion of SV40 Intron into polymerase coding sequence

Splice Donor Site Splice Acceptor Site
 ↓ ↓
 (D) -----UGCUCUCUAAGGUAAAUAU - - - - - UGUUUUUUAGGGUCUACUC-----
 mRNA transcript containing intron

(E) -----UGCUCUCUAAGGGUCUACUC-----
 mRNA transcript after splicing has normal T7 Sequence

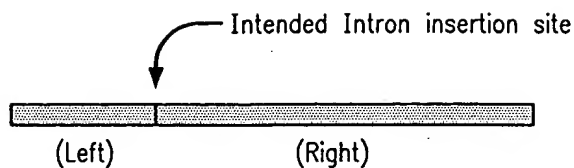
FIG. 24

Fusion of Intron into T7 RNA Polymerase Coding Sequence

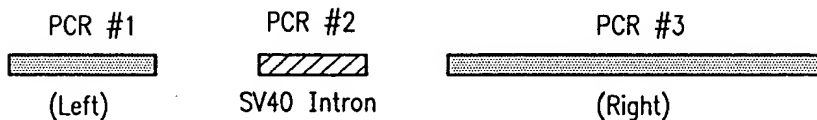
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(A)

Normal T7 RNA polymerase coding sequence

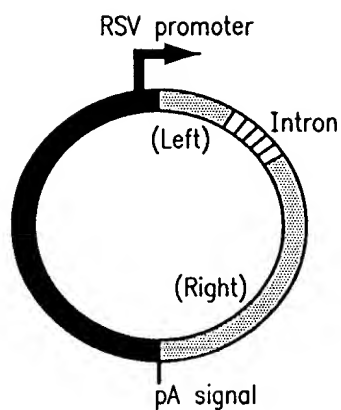


Synthesis of fragments by PCR Amplification of T7 or SV40 templates



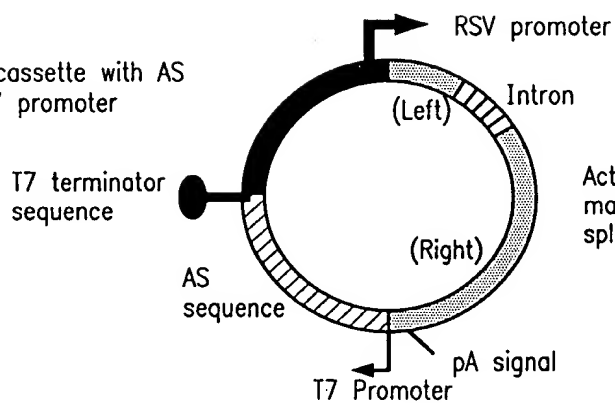
(B)

Fusion of PCR fragments together in eucaryotic expression vector



(C)

Introduction of cassette with AS directed from T7 promoter



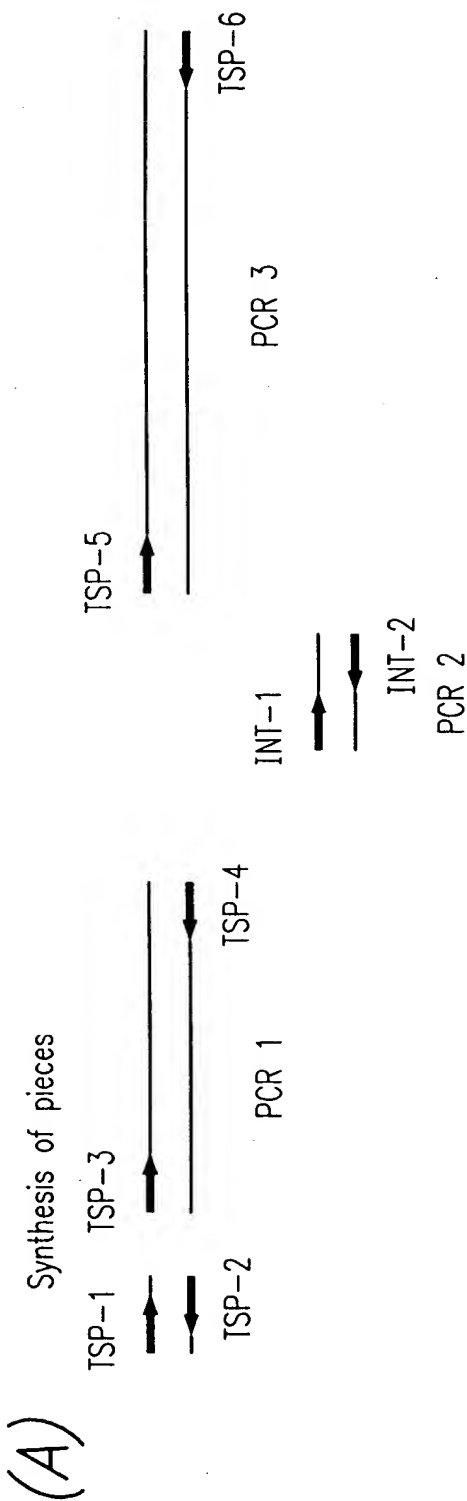
Active T7 RNA polymerase is only made in eucaryotic cells after splicing out of SV40 Intron

FIG. 25

Construction of T7 Expression Vector



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(B) Oligomers used for synthesis

TSP-1	GGA ATT CGT CTC GAG CTC TGA TCA CCA CCA TGG ACA CGA TTA ACA TCG C
TSP-2	GAC TAG TTG GTC TCG TCT CTT TTT TGG AGG AGT GTC GTT CTT AGC GAT GTT AAT C
TSP 3	GGA ATT CGT CTC GGA GAA AGG TAA AAT TCT CTG ACA TCG AAC TGG C
TSP-4	GAC TAG TGG TCT CCC CTT AGA GAG CAT GTC AGC
TSP-5	GGA ATT CGG TCT CGG GTC TAC TCG GTG GCG AGG
TSP-6	GAC TAG TCG TTA CGC GAA CGC AAA GTC
INT-1	GGA ATT CGT CTC TAA GGT AAA TAT AAA ATT TTT AAG
INT-2	GAC TAG TCG TCT CTG ACC CTA AAA TAC ACA AAC AAT TAG A

FIG. 26

Synthesis of Pieces for Construction of
T7 RNA Polymerase with Intron



TSP1
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GC 3'
3' C TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG 5'
TSP2

Extension of TSP1/TSP2 by polymerase
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA CTC 3'
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG 5'
Bsa I

Digestion of TSP1/TSP2 product with Bsa I
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AA
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT

Digestion of PCR #1 clone (pL-1) with BsmB I
Bsm B1
5' GGA ATT CGT CTC G GAGA AAG GTA AAA TTC TCT GAC ATC GAA CTG GC-----
CCT TAA GCA GAG CCTCT TTC CAT TTT AAG AGA CTG TAG CTT GAC CG-----

Ligation of Bsa I digested TS1/TS2 product to BsmB I digested PCR#1 clone
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA AAG GTA AAA TTC
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT TTC CAT TTT AAG
TCT GAC ATC GAA CTG GC-----
AGA CTG TAG CTT GAC CG-----

FIG. 27
Formation of Nuclear Localisation Signal by Fusion of TSP1/TSP2 Product to
Clone with PCR #1 product

Wild Type T7 nucleic and amino acid sequence

ATG	GAC	ACG	ATT	AAC	ATC	GCT	AAG	AAC	GAC	TTC	TCT	GAC	ATC	GAA	CTG	GC	-----
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTC	TTG	CTG	AAG	AGA	CTG	TAG	CTT	GAC	CG	-----
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		

Modified T7 nucleic and amino acid sequence
with Nuclear Localisation Signal (NLS) insertion

ATG	GAC	ACG	ATT	AAC	ATC	GCT	AAG	AAC	GAC	ACT	CCT	CCA	AAA	AAG	AGA	GTA	AAA	TTC	TCT	GAC	ATC	GAA	CTG	GC	-----	
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTC	TTG	CTG	TGA	GGA	GGT	TTT	TTT	CTC	TTC	TTC	TTC	TTC	AGA	CTG	TAG	CTT	GAC	CG	-----
1	2	3	4	5	6	7	8	9	10																	

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FIG. 28

Comparison of the 5' ends of the Nucleotide Sequences of Wild Type
and Modified T7 RNA Polymerase

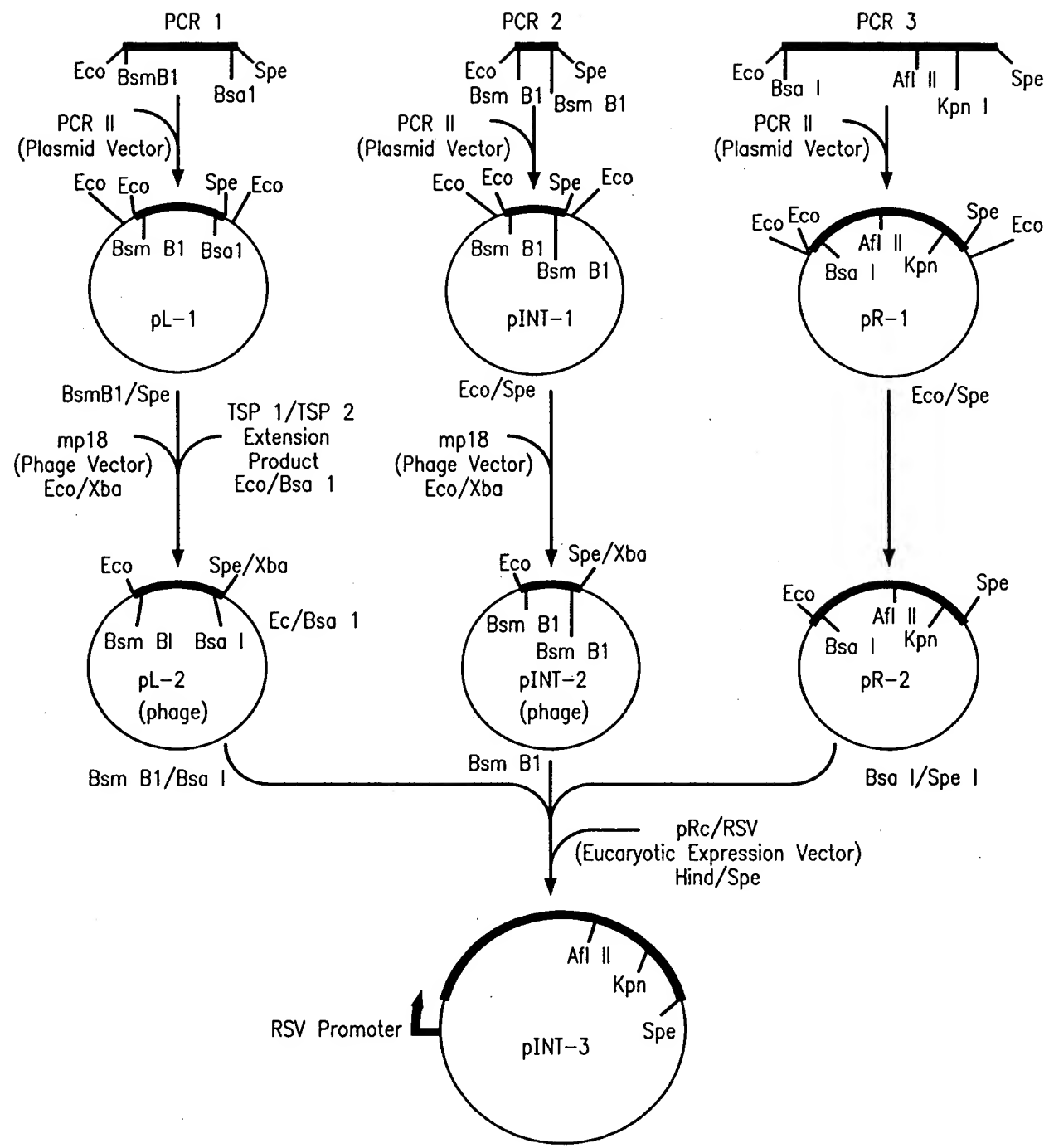


FIG. 29

Fusion of PCR Pieces to Construct
T7 RNA Polymerase with an Intron

(A) Oligomers

HTA-1 GAT CAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAG
HTA-2 GAT CCT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT

HTB-1 GAT CAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA G
HTB-2 GAT CCT TGA GGA GGT CTT CGT CGC TGT CTC CGC TTC TTC CTG CCA TAG GAG AGC CTA AGG T

HTC-1 GAT CAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GGT TCA GAC CCA CCT CCC AG
HTC-2 GAT CCT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT

TER-1 AAT CTA GAG CTA ACA AAG CCC GAA AGG AAG
TER-2 TTC TGC AGA TAT AGT TCC TCC TTT CAG C

(B) Cloning of AS and Terminator sequences into vector with T7 Promoter

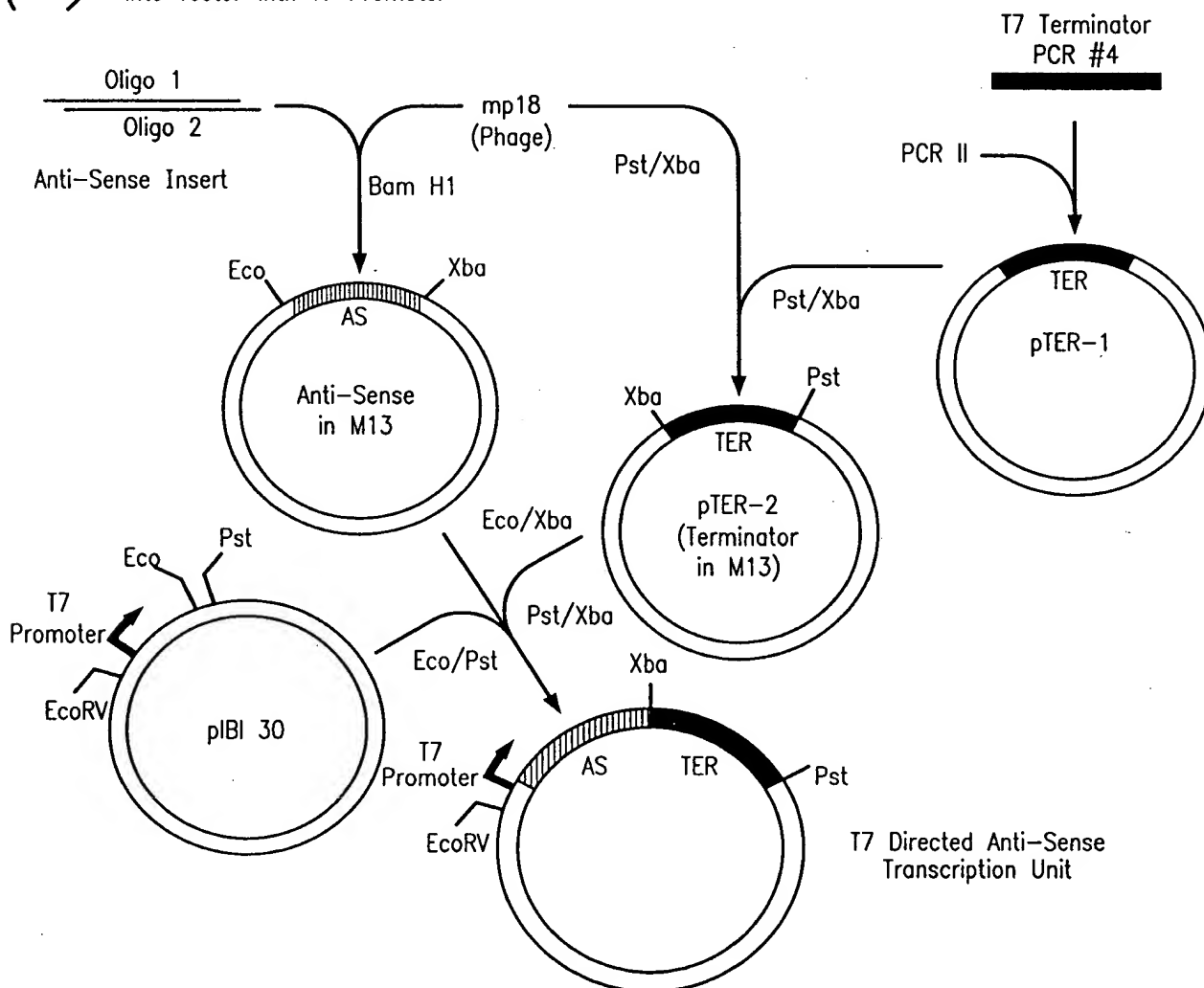


FIG. 30

Insertion of Anti-Sense Sequences into
T7 Directed Transcription Units

Construct with t7 RNA polymerase and
Anti-Sense directed from a T7 Promoter



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A) Oligomers for introduction of T7 signals and polylinker

PL-1 TCG AGC CAT GGC TTA AGG ATC CGT ACG TCC GGA GCT AGC GGG CCC ATC GAT ACT
AGT TAA ATG CAG ATC T

PL-2 CTA GAG ATC TGC ATT TAA CTA GTA TCG ATG GGC CCG CTA GCT CCG GAC GTA CGG
ATC CTT AAG CCA TGG C

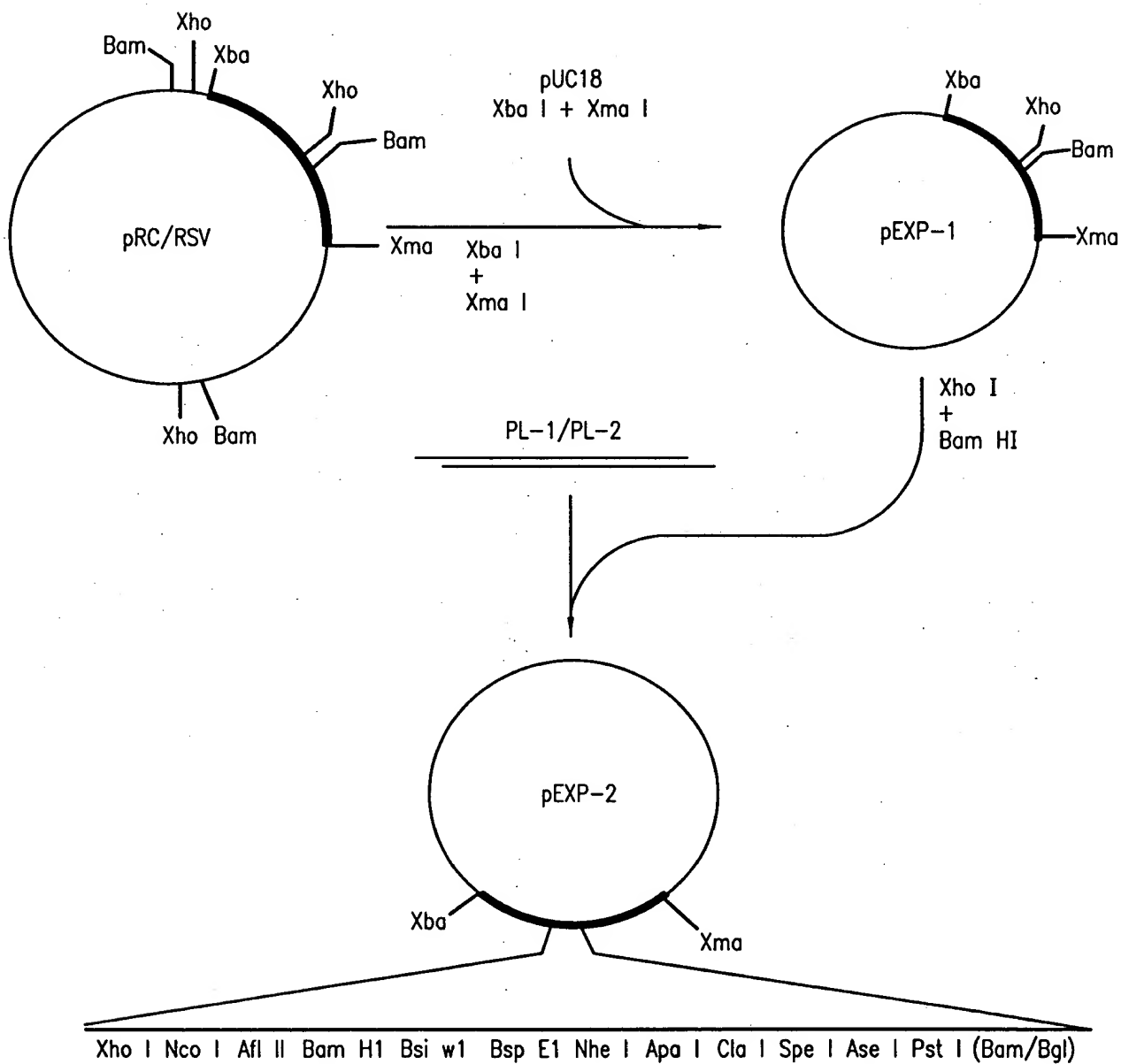


FIG. 32

Introduction of Poly-Linker for Creation of Protein Expression Vector

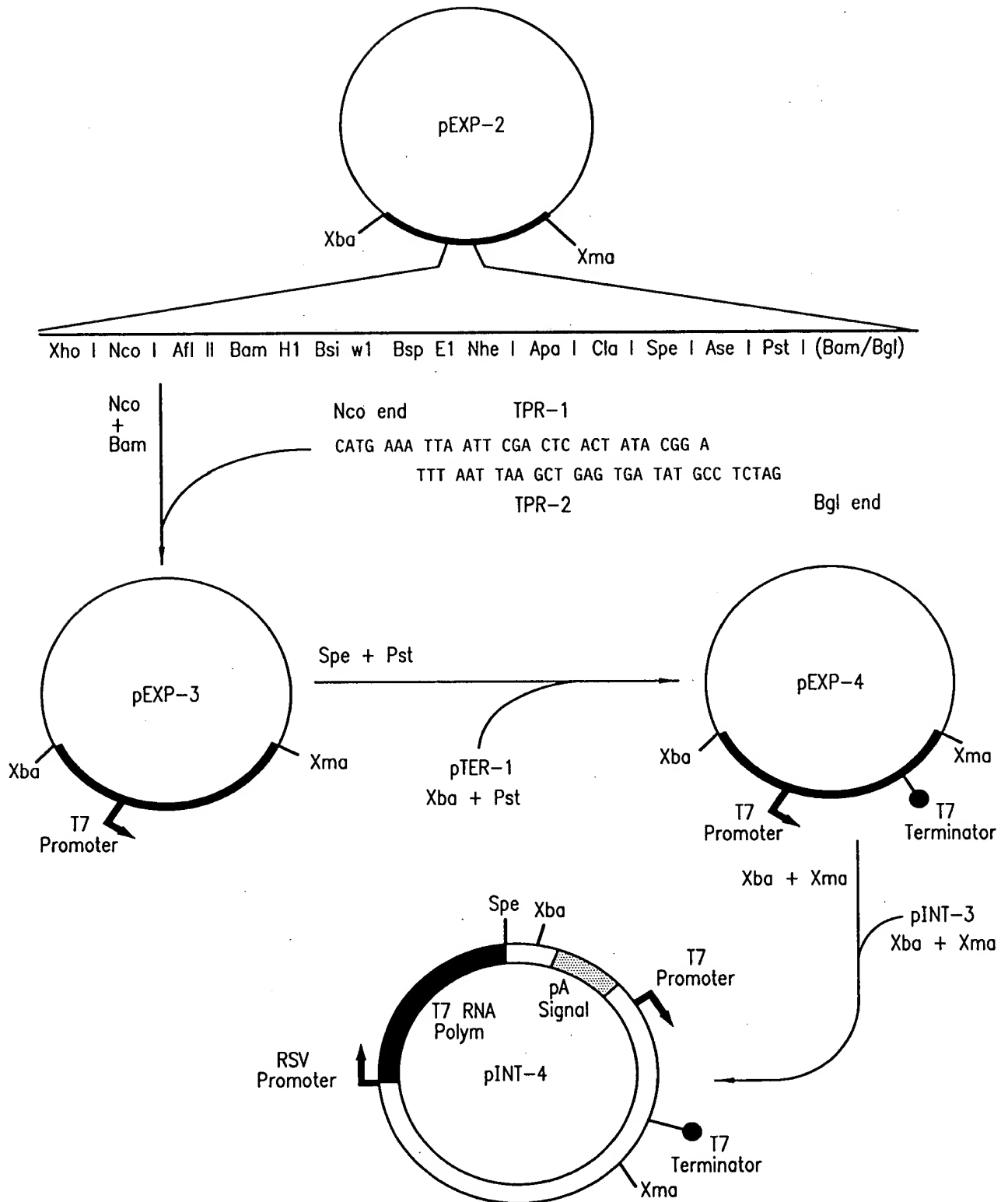


FIG. 33

Final steps for construction of Expression Vector

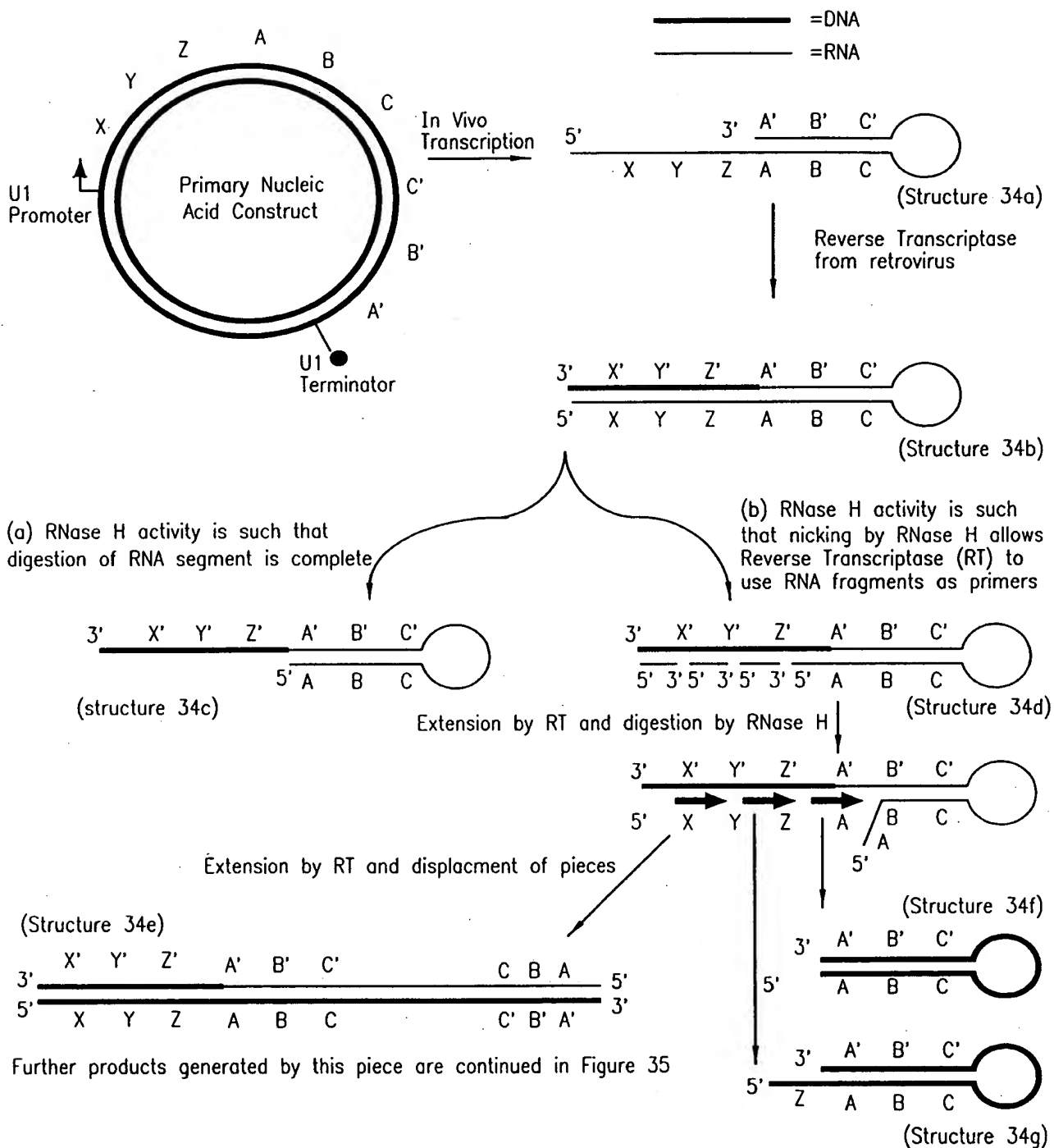


FIG. 34

Construct that produces single-stranded Anti-Sense DNA

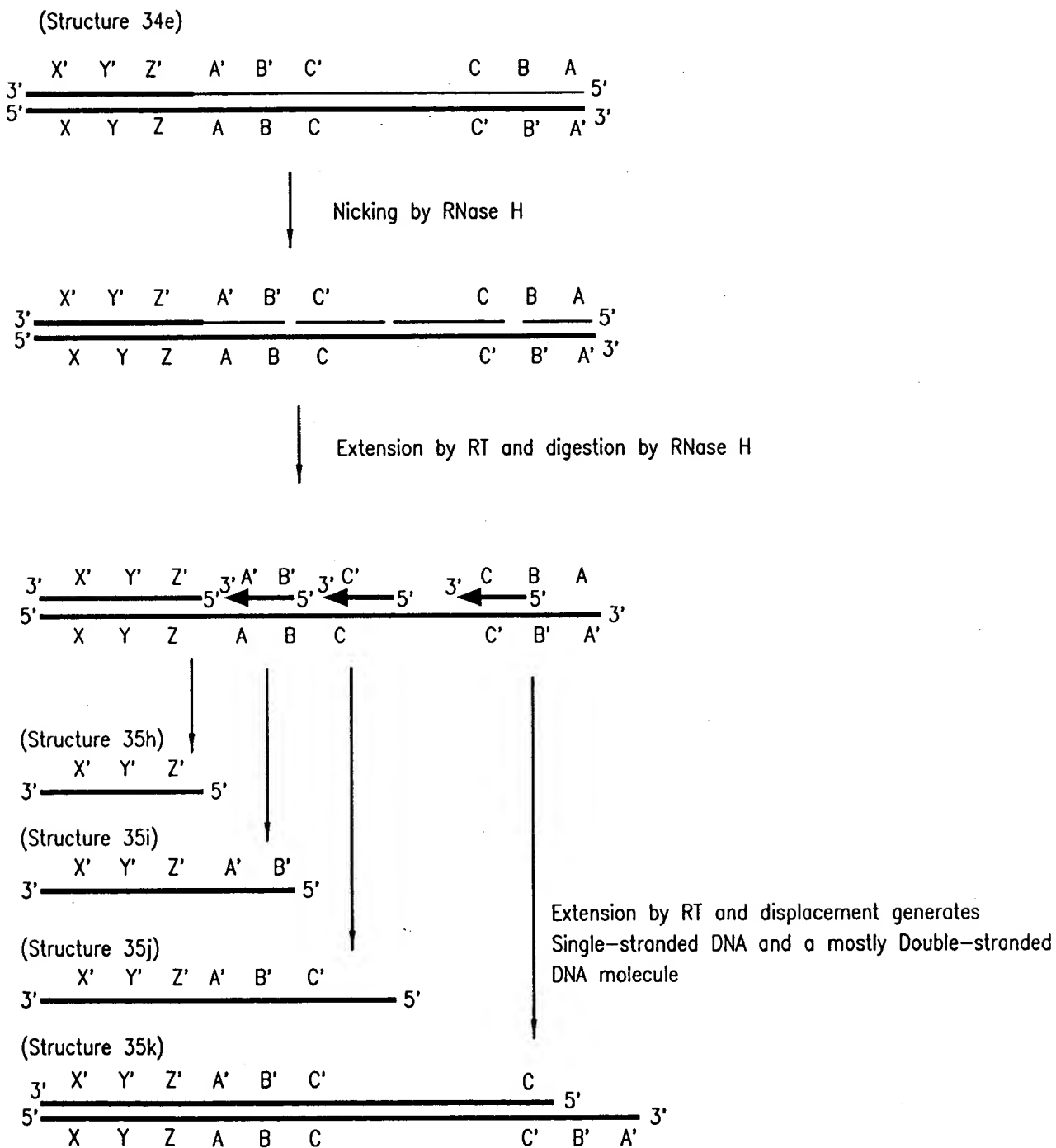


FIG. 35

Continuation of Process from Figure 34

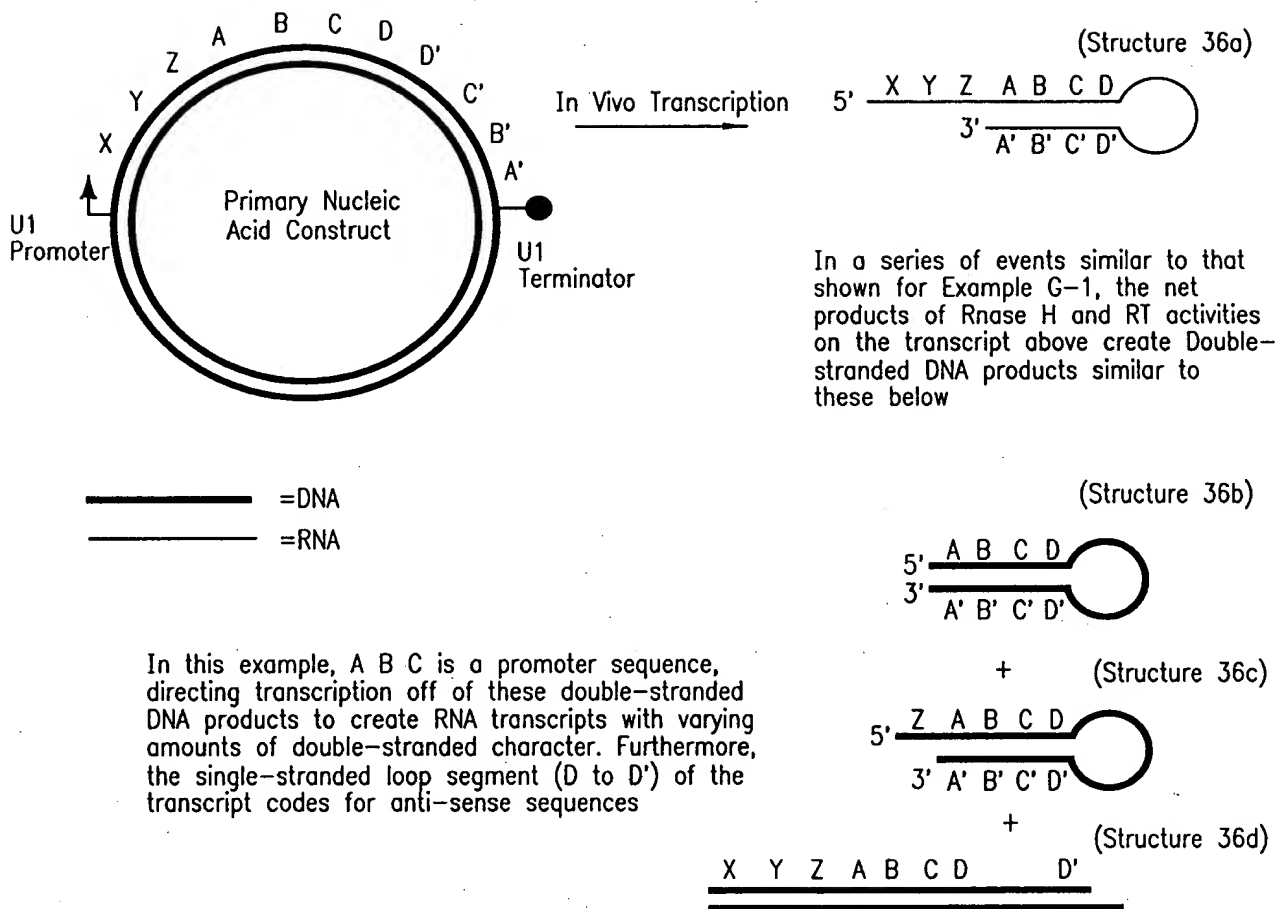


FIG. 36

Construct that produces RNA that is Reverse Transcribed to create Secondary DNA Constructs capable of directing transcription

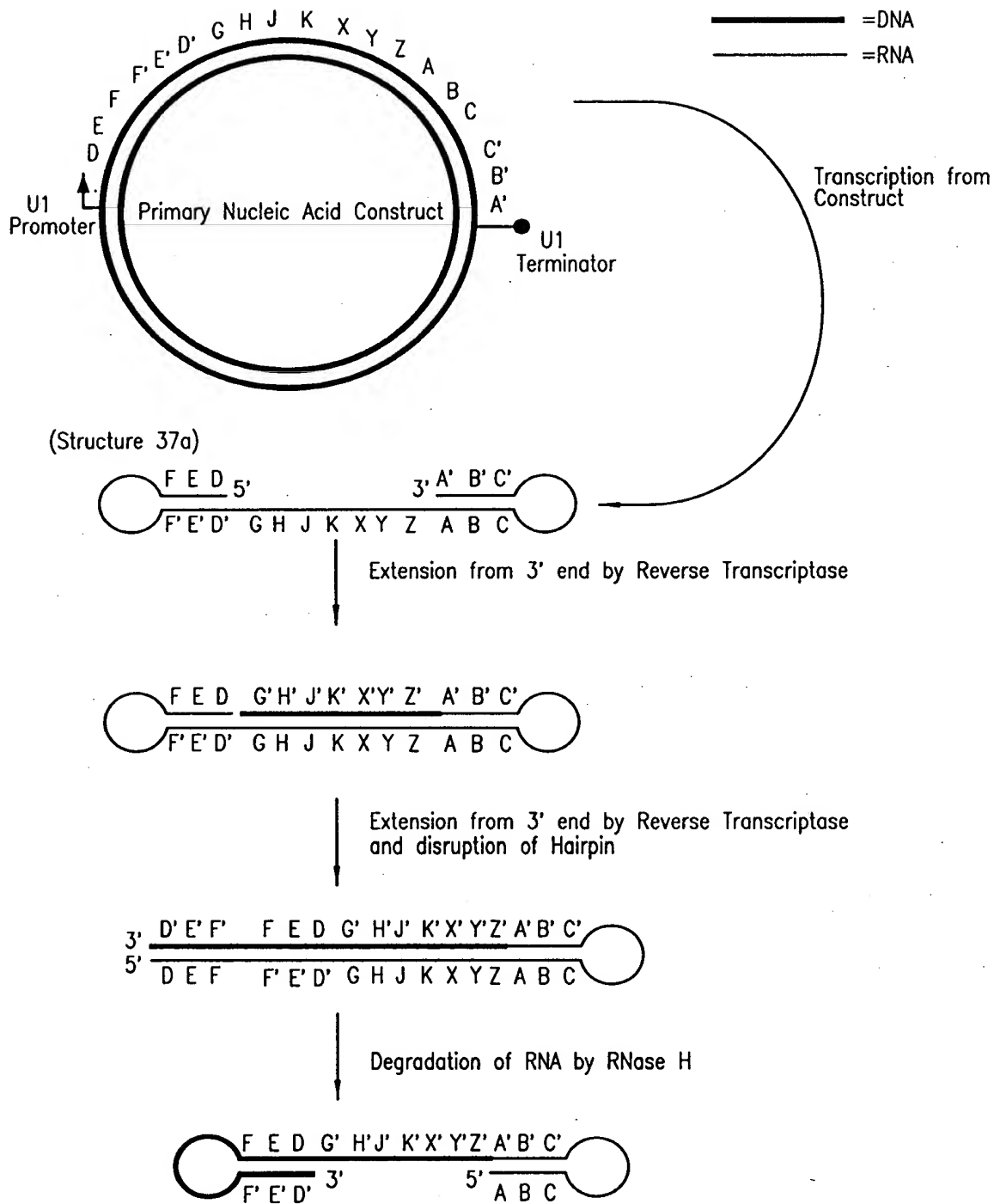
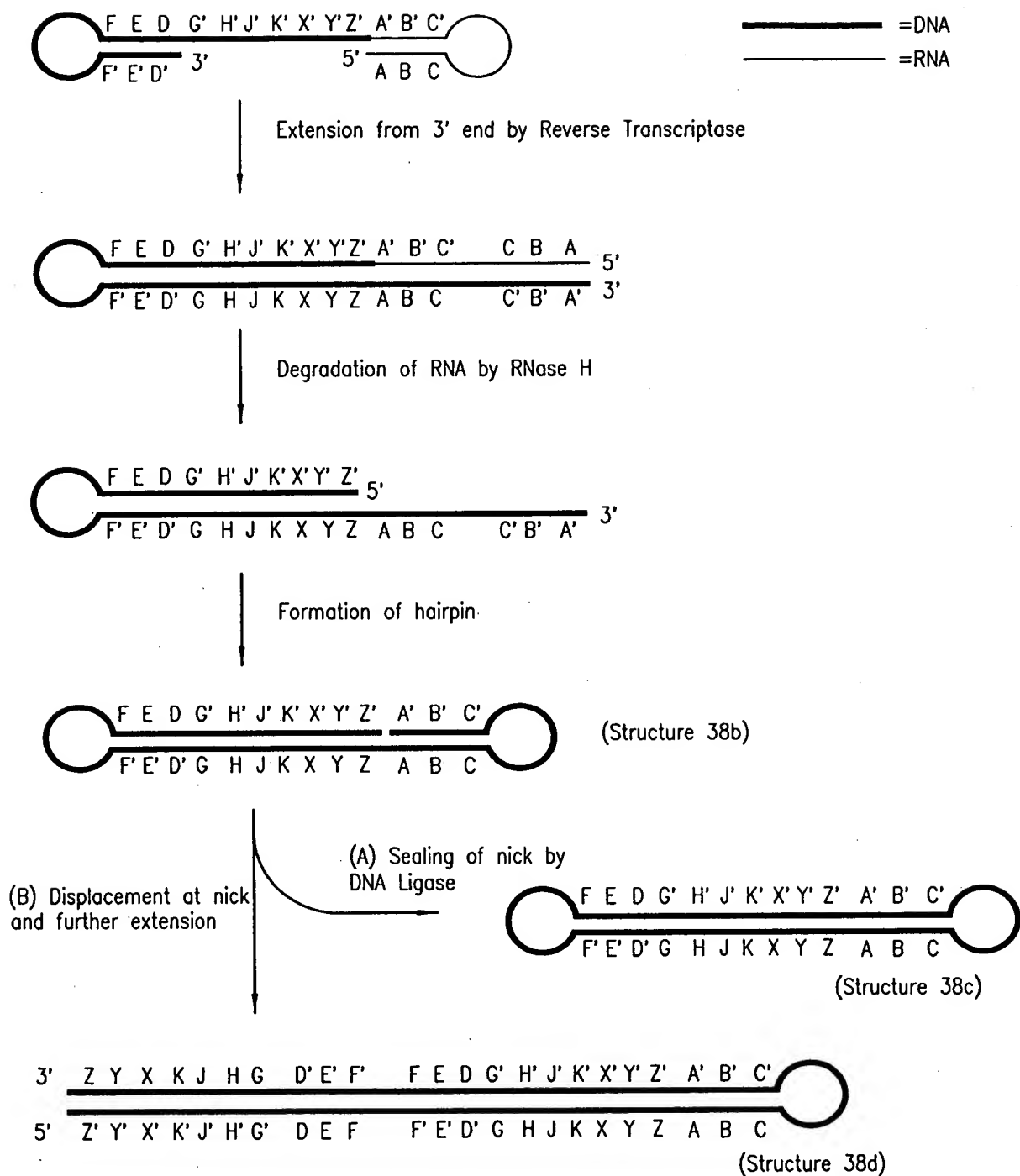


FIG. 37

Construct which Propagates a Double Hairpin Production Center



In this Example, the sequence F' E' D' is a promoter, the sequence GHJK is an Anti-Sense sequence and X Y Z is a poly A signal

FIG. 38

Continuation of process from Figure 37

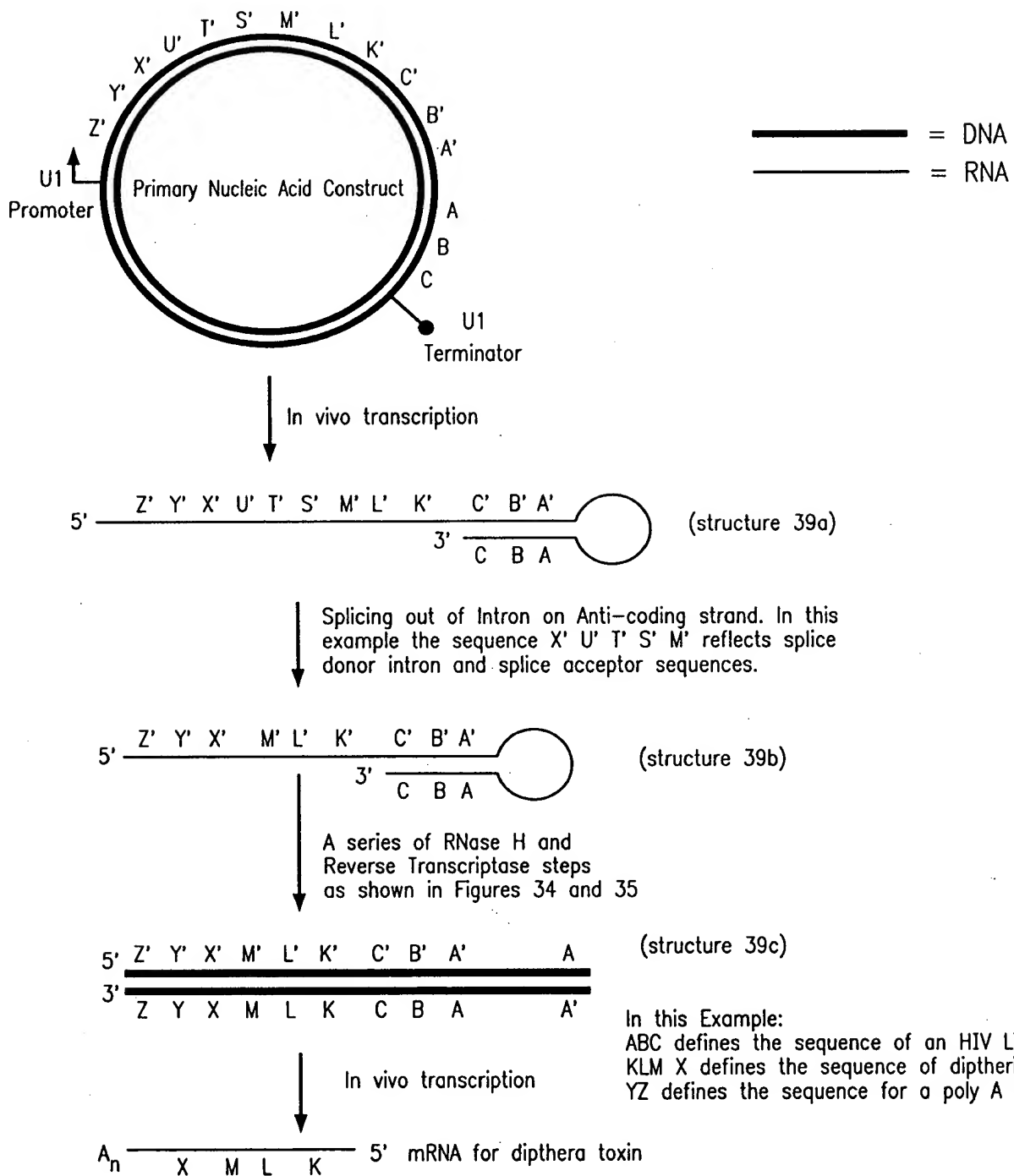


FIG. 39

Construct which propagates a Production Center capable of Inducible Suicide

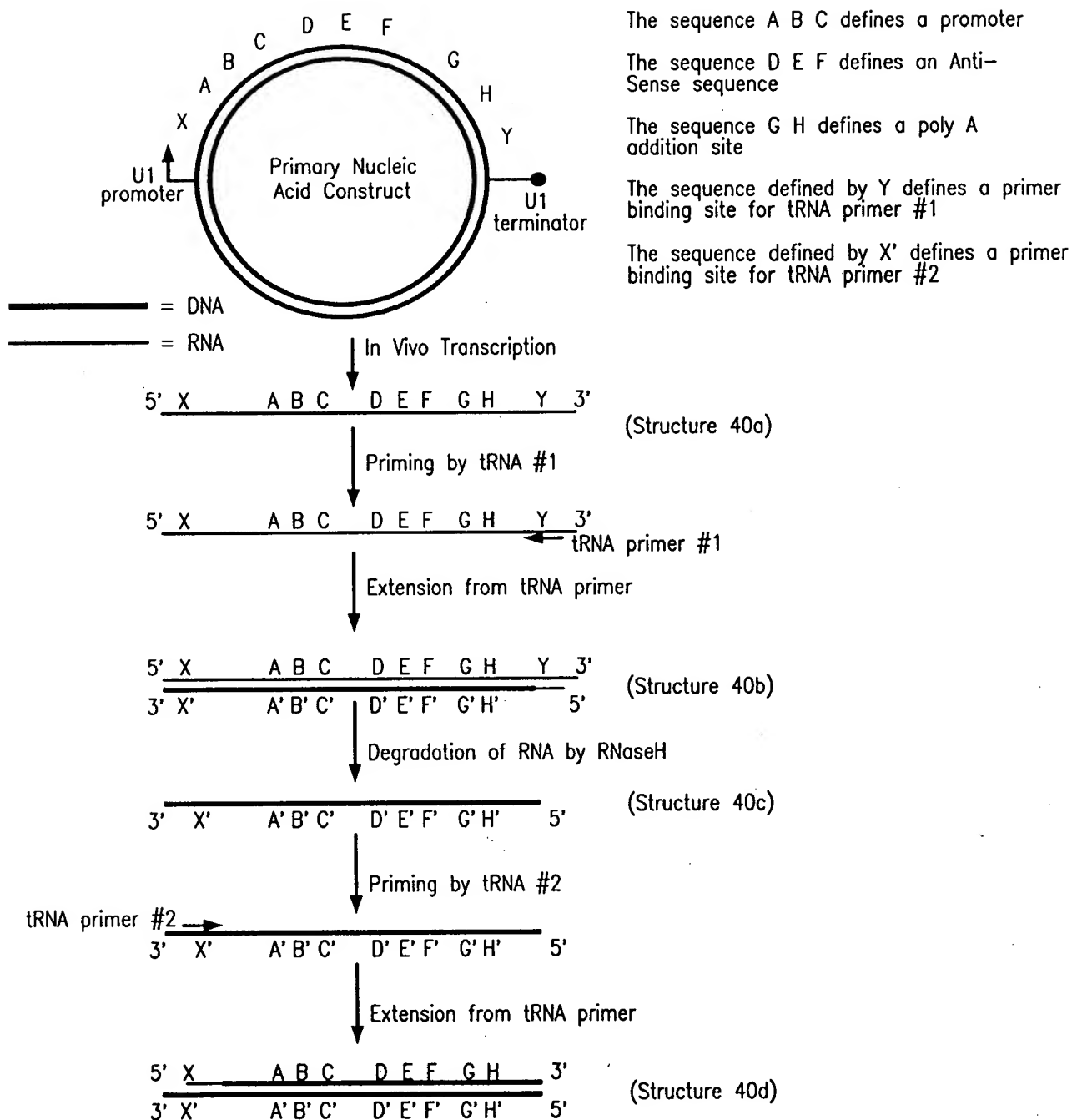


FIG. 40

Use of tRNA primers to create a DNA construct
for secondary production of transcripts

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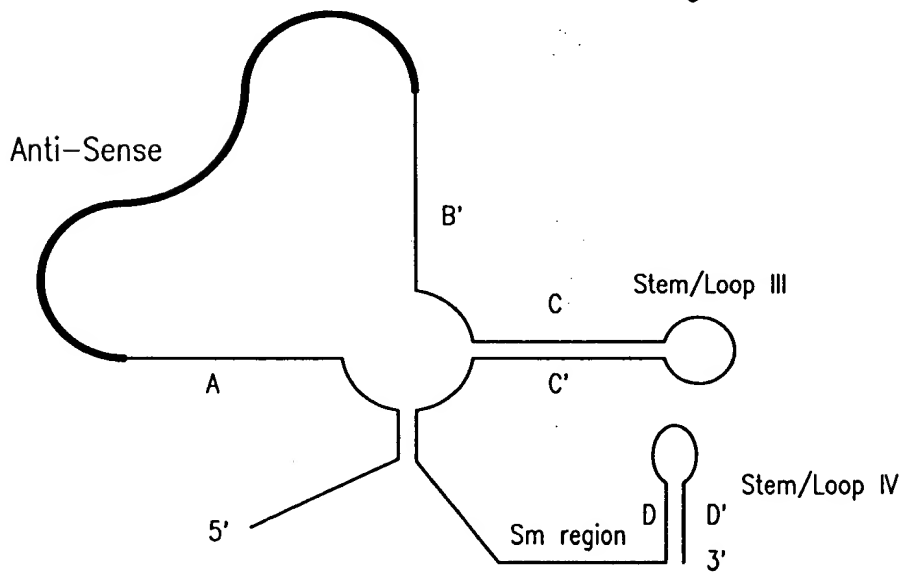
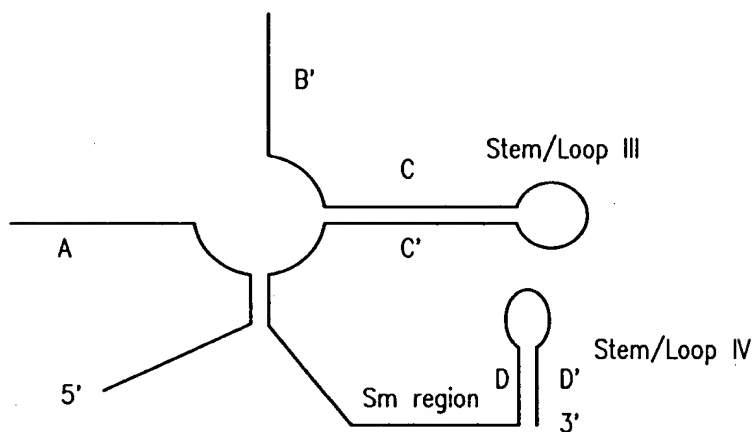
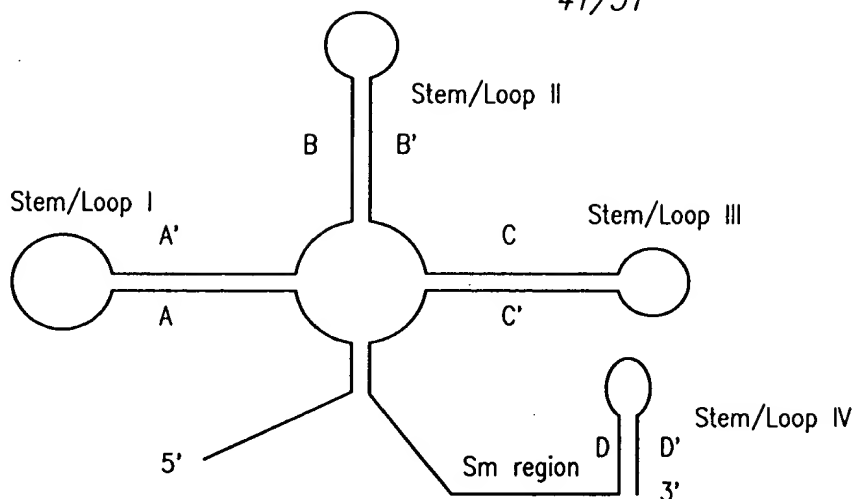


FIG. 41

Excision of sequences from U1 Transcript Region
and Replacement with Novel Sequences

(A) Anti-sense oligomers

HVA-1 GAT CCG GAT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT
HVA-2 CCG GAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAT CCG
HVB-1 GAT CCG GAC CTT GAG GAG GTC TTC GTC GCT GTC TCC GCT TCT TCC TGC CAT AGG AGA GCC TAA GGT
HVB-2 CCG GAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGT CCG
HVC-1 GAT CCG GAT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT
HVC-2 CCG GAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC ATC CG
HVD-1 GAT CAG CAT GCC TGC AGG TCG ACT CTA GAC CCG GGT ACC GAG CTC GCC CTA TAG TGA GTC GTA TTA T
HVD-2 CCG GAT AAT ACG ACT CAC TAT AGG GCG AGC TCG GTA CCC GGG TCT AGA GTC GAC CTG CAG GCA TGC T

(B) Replacment of U1 sequences with HIV Anti-sense sequences

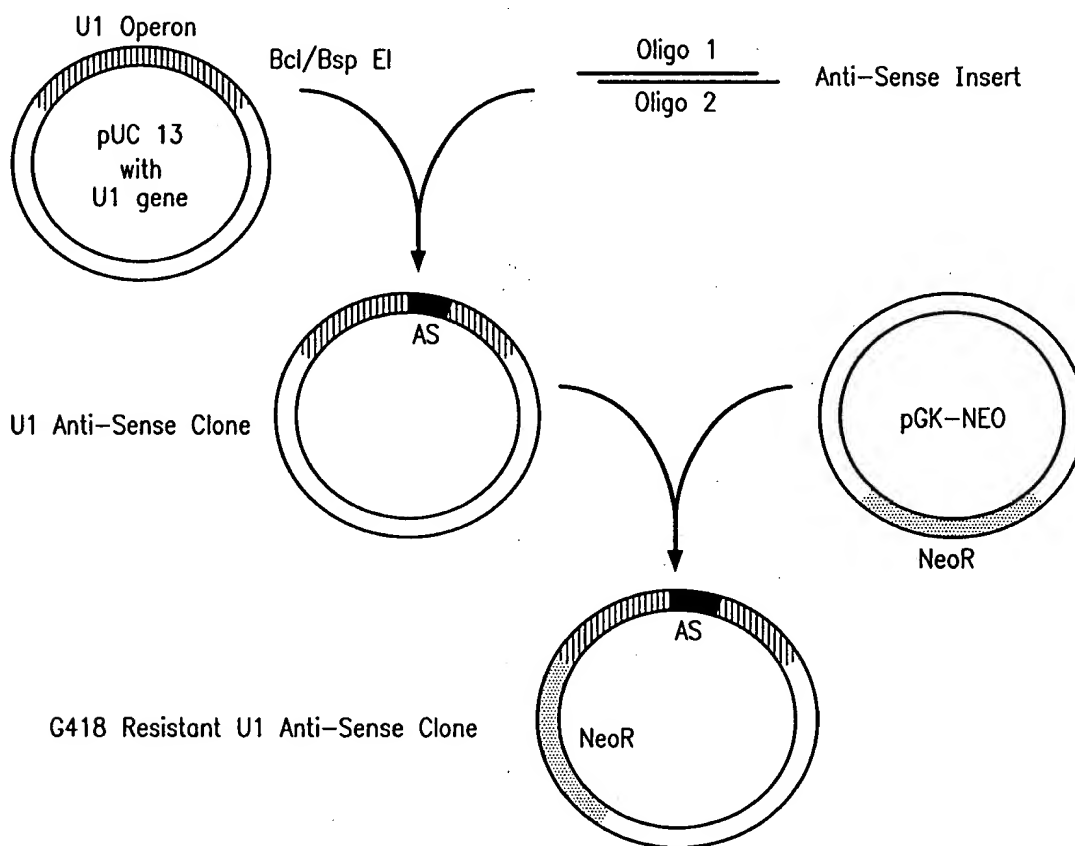


FIG. 42

Insertion of Anti-Sense Sequences into U1 Operons

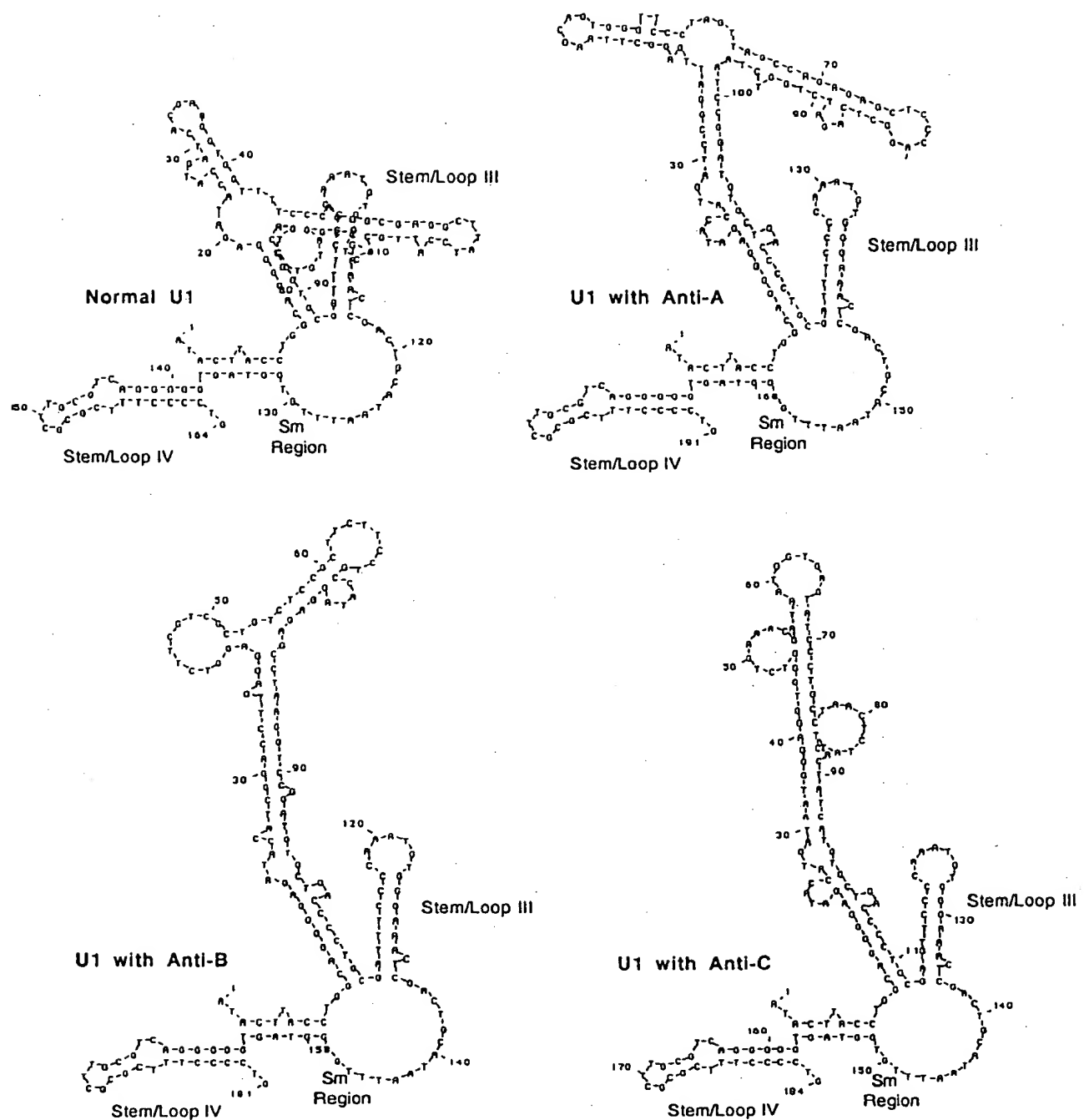


FIG. 43

Predicted secondary structures for U1
Transcripts with Anti-sense Substitutions

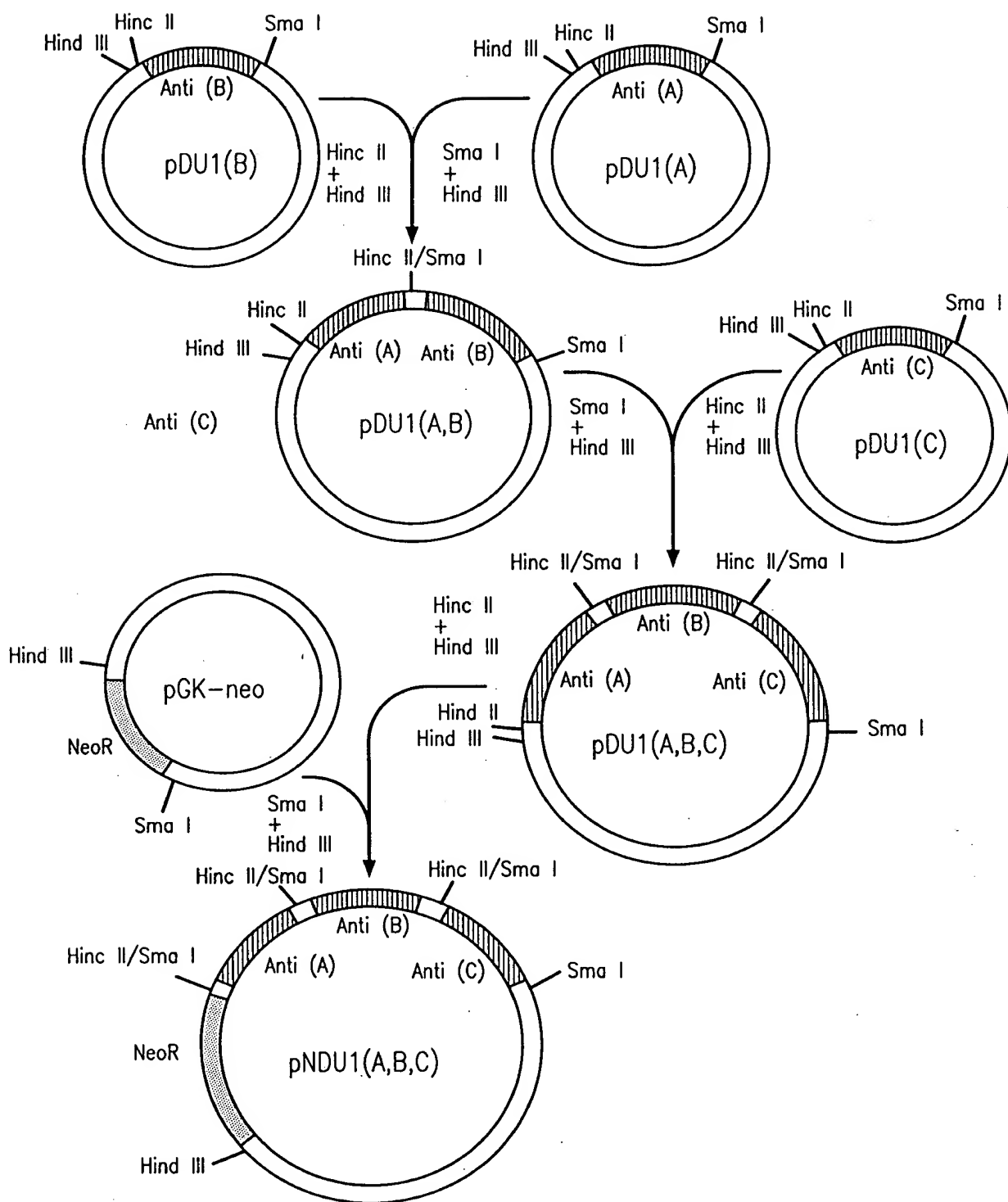


FIG. 44

Construction of U1 Multiple Operon Clone

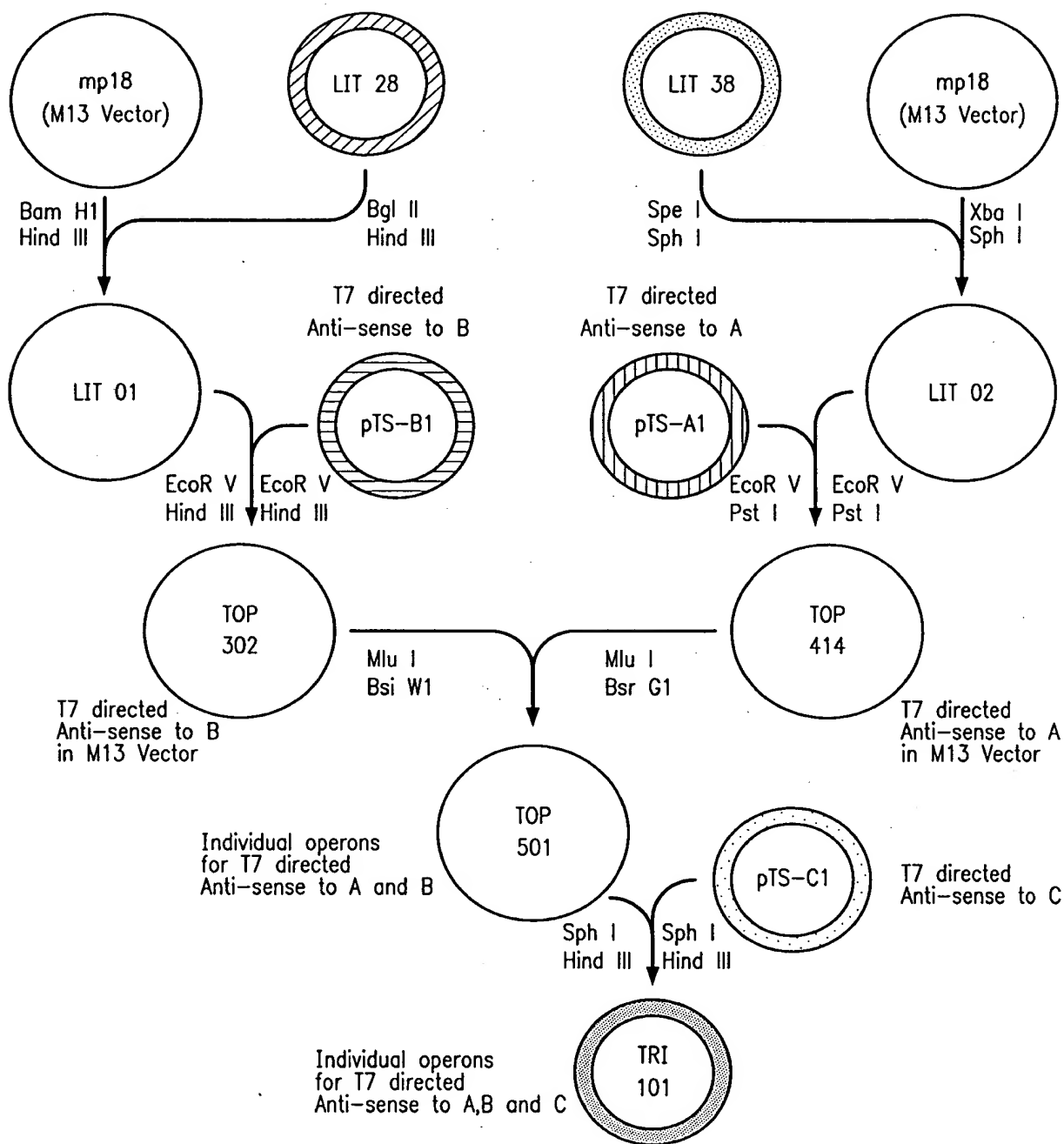
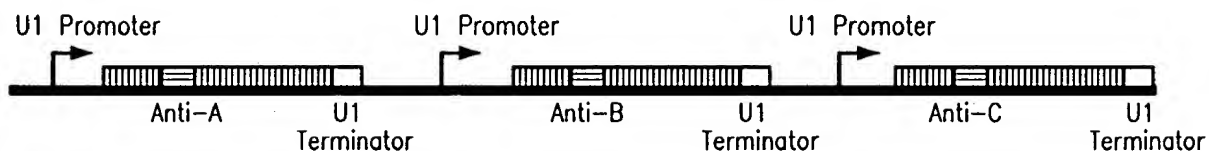


FIG. 45
Construction of T7 Triple Operon

pNDU1(A,B,C)

Triple U1 Operon Construct with HIV Anti-Sense



TRI 101

Triple T7 Operon Construct with HIV Anti-Sense

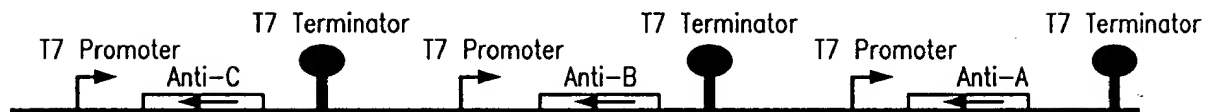


FIG. 46

Structures of Triple Operon Constructs
 from Figures 44 and 45

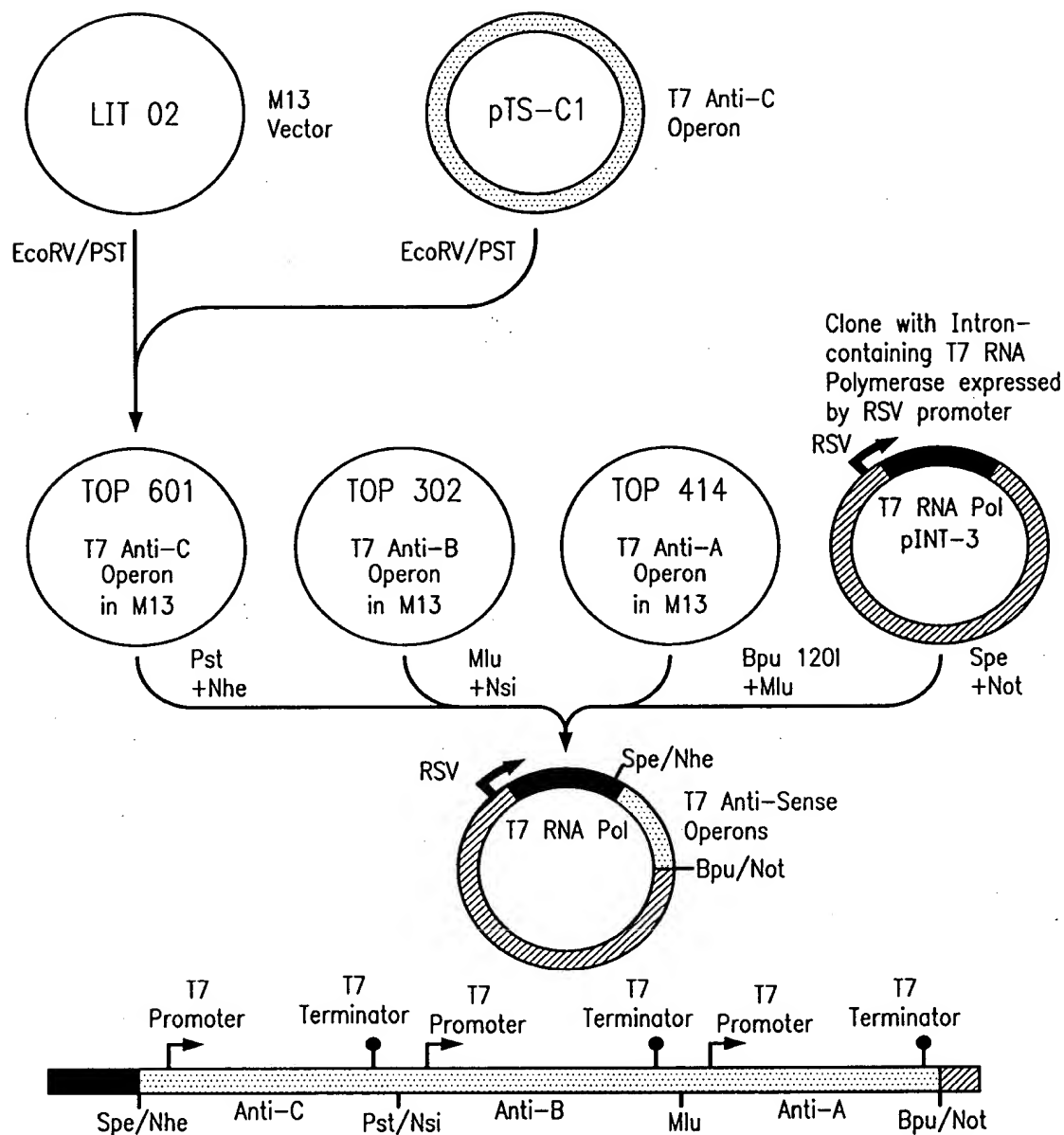
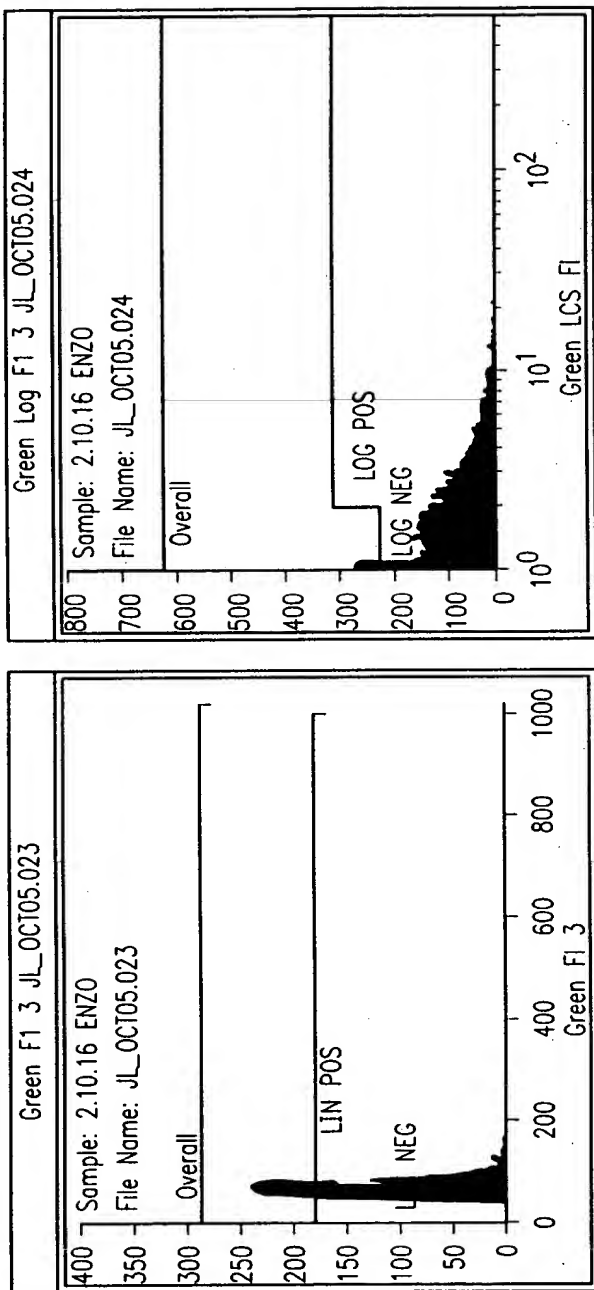


FIG. 47

Construction of Multiple T7 Operons in
Vector coding for T7 RNA Polymerase



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Global Statistics									
1. Green F1 3 JL_OCT05.023		Total = 7509							
2. Green Log FL JL_OCT05.024		Total = 7509							
Hist	Region	Bounds	Counts	*	Mean	X	Mean	Y	Made xc
1.	LIN NEG	1 78	5714	76.1	63.65			78	14
	LIN POS	85 1002	1129	15.0	97.34			85	17
	OVERALL	1 1024	7509	100.0	70.28			70	23
2.	LOG NEG	2 2	4211	56.1	2.34			2	21
	LOG POS	2 1001	3407	45.4	4.76			3	69
	OVERALL	2 1001	7509	100.0	3.43			2	88

FIG. 48

Flow cytometry data measuring binding of anti -CD4+ antibody to HIV resistant U037 cells



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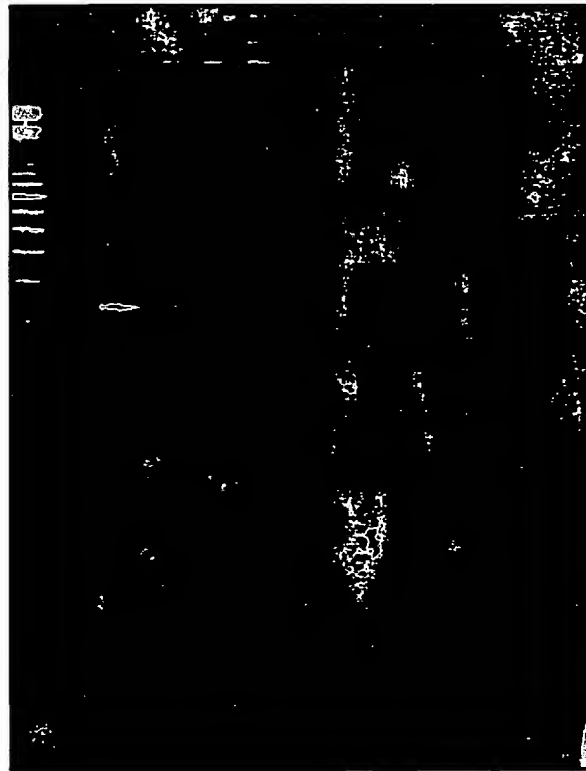


FIG. 49

PCR amplification of gag region
indicating absence of HIV in
viral resistant cell line (2.10.16)
after challenge

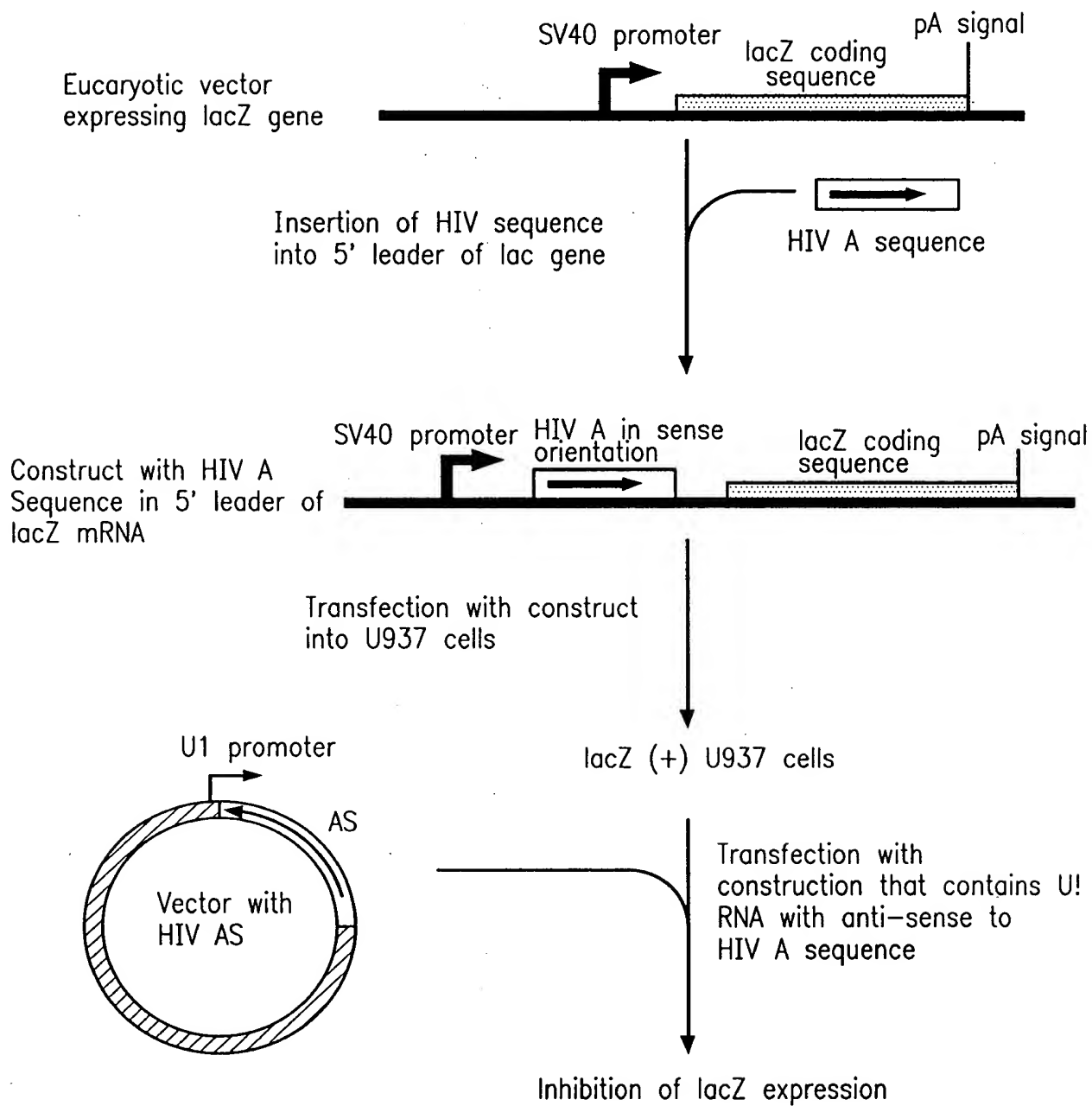


FIG. 50

Clone with target-lacZ fusion will have reduced expression of lacZ after transfection by HIV Anti-sense construct



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Enzyme activity as expressed by A_{420} readings
in extracts prepared from

(A)

	2.5×10^4 cells	5×10^4 cells	1.0×10^5 cells
U 937 (untransfected)	0.018	0.023	0.034
U 937 (HIV A clone)	0.154	0.277	0.566
U937 (HIV A/Anti-A)	0.010	0.017	0.027
U 937 (HIV A/Anti-ABC)	0.013	0.021	0.035
U 937 (HIV A/Null DNA)	0.120	0.212	0.337

(B)

Expression of Beta-galactosidase activity by In situ assay:

U 937 (untransfected)	no blue spots in cells
U 937 (HIV A clone)	blue spots in cells
U 937 (HIV A/Anti A)	no blue spots in cells
U 937 (HIV A/Anti ABC)	no blue spots in cells
U 937 (HIV A/Null DNA)	blue spots in cells

FIG. 51

Expression of Beta-galactosidase activity
in extracts